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MARTINEZ-MARTIN et al.

(54) **BIOLOGICAL VESICLES DISPLAYING CELL SURFACE PROTEINS AND METHODS RELATED TO SAME**

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Related U.S. Application Data

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(60) Provisional application No. 63/227,039, filed on Jul. 29, 2021, provisional application No. 63/212,021, filed on Jun. 17, 2021, provisional application No. 63/120,167, filed on Dec. 1, 2020.

Publication Classification

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G01N 33/68 (2006.01)

(52) **U.S. Cl.**
CPC ***G01N 33/6845*** (2013.01)

(57) **ABSTRACT**

Provided herein are biological vesicles displaying cell surface proteins, as well as methods of using such vesicles to identify and characterize protein-protein interactions.

Specification includes a Sequence Listing.

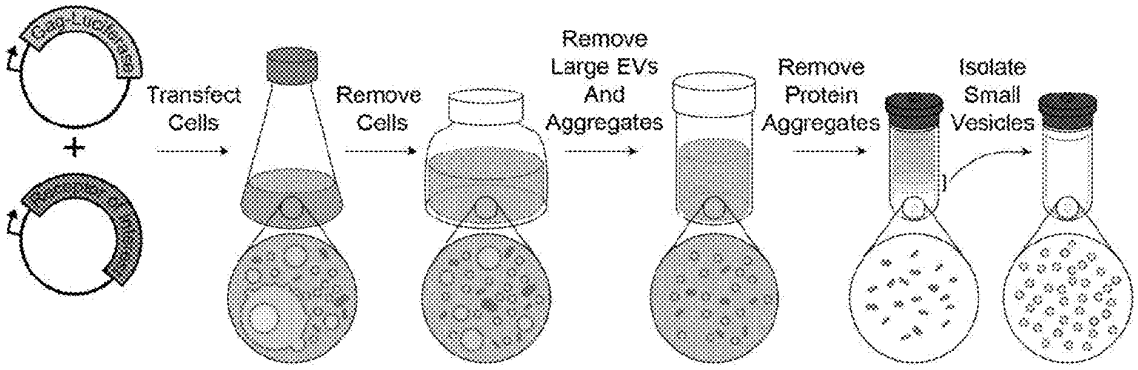
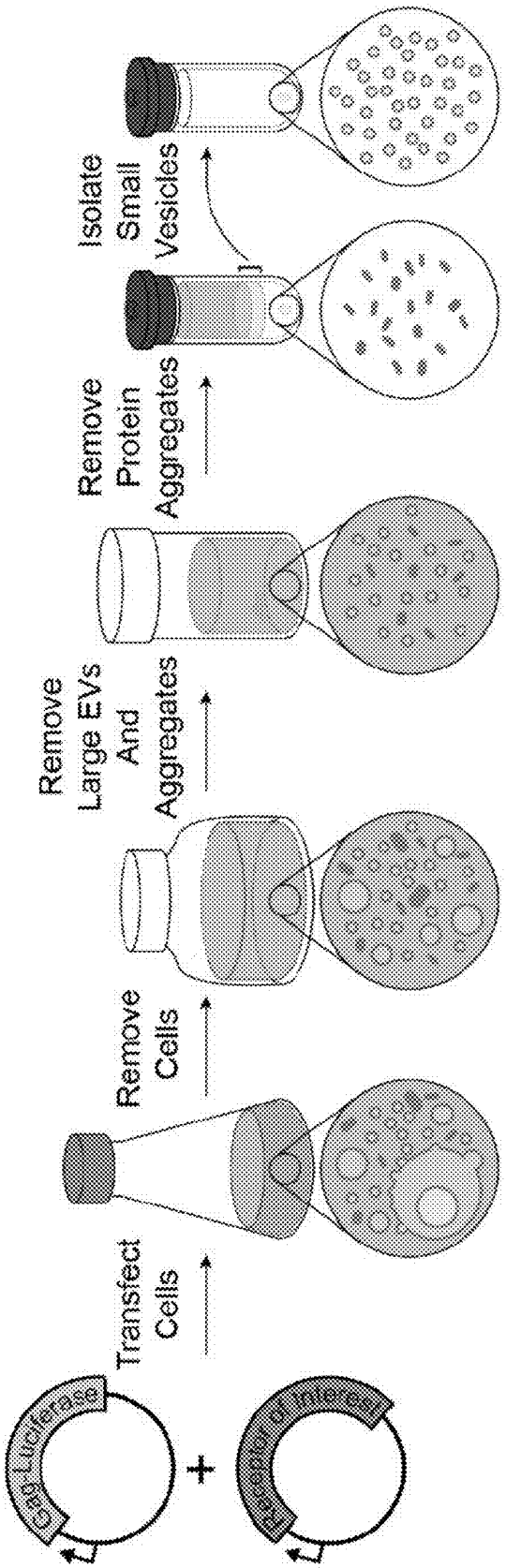


FIG. 1A


Cap Luciferase
+
Transfect Cells
Remove Cells
Remove Large EVs And Aggregates
Remove Protein Aggregates
Isolate Small Vesicles

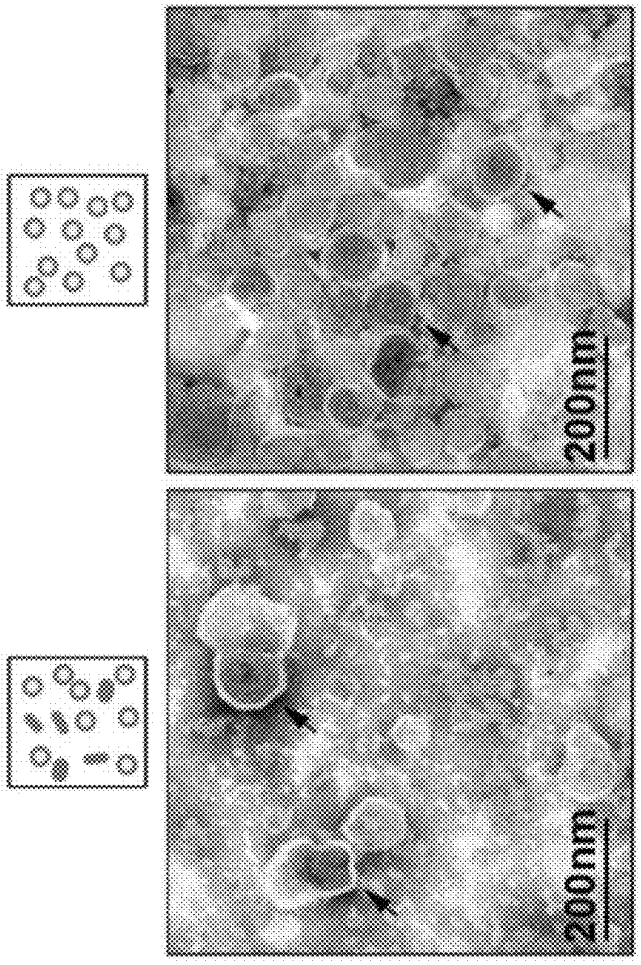


FIG. 1B

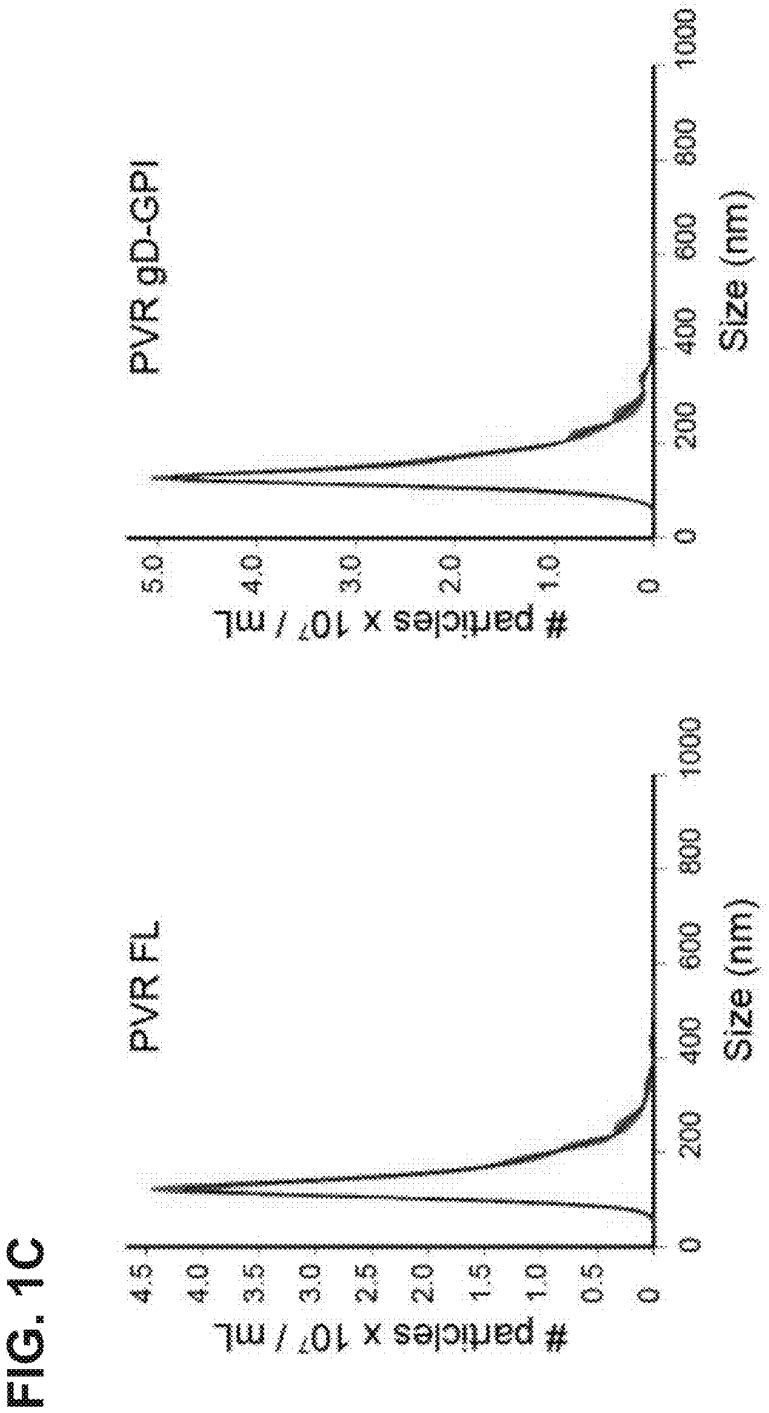

PVR gD-GPI
particles x 10⁷ / mL
5.0
4.0
3.0
2.0
1.0
0
0
200
400
600
800
1000
Size (nm)
PVR FL
particles x 10⁷ / mL
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
0
200
400
600
800
1000
Size (nm)

FIG. 1C

FIG. 2A

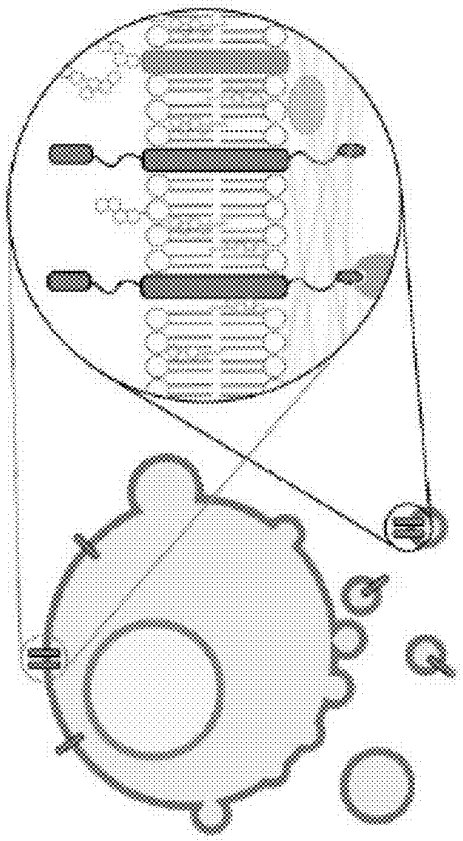

Q
Q

FIG. 2B

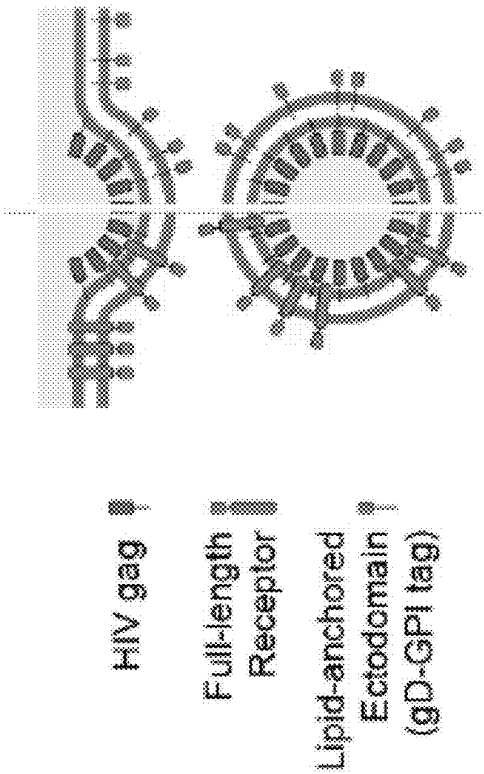

HIV gag
Full-length Receptor
Lipid-anchored Ectodomain (gD-GPI tag)

FIG. 2D

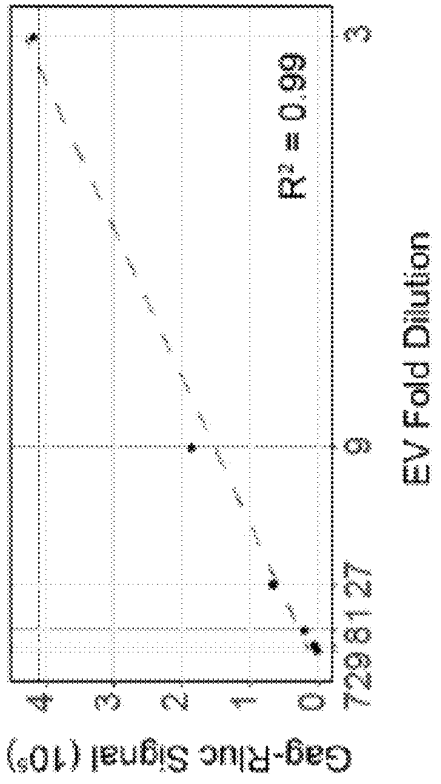

Gag-Rluc Signal (10⁵)
0
1
2
3
4
729
81
27
9
3
EV Fold Dilution
R² = 0.99

FIG. 2C

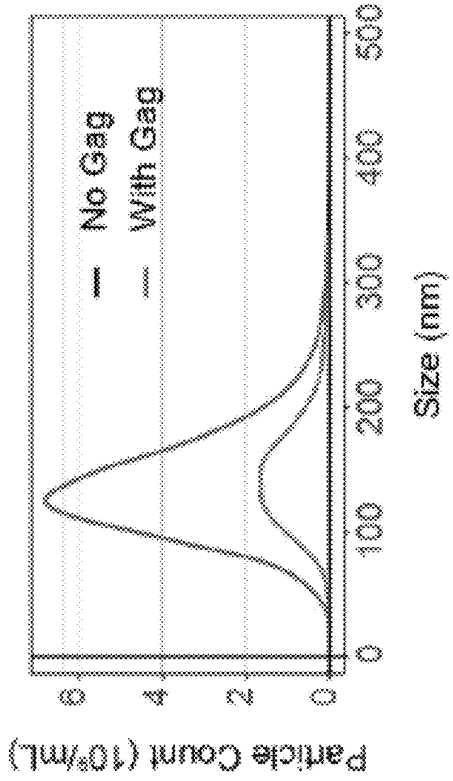

Particle Count (10⁹/mL)
0
2
4
6
0
100
200
300
400
500
Size (nm)
No Gag
With Gag

FIG. 2E

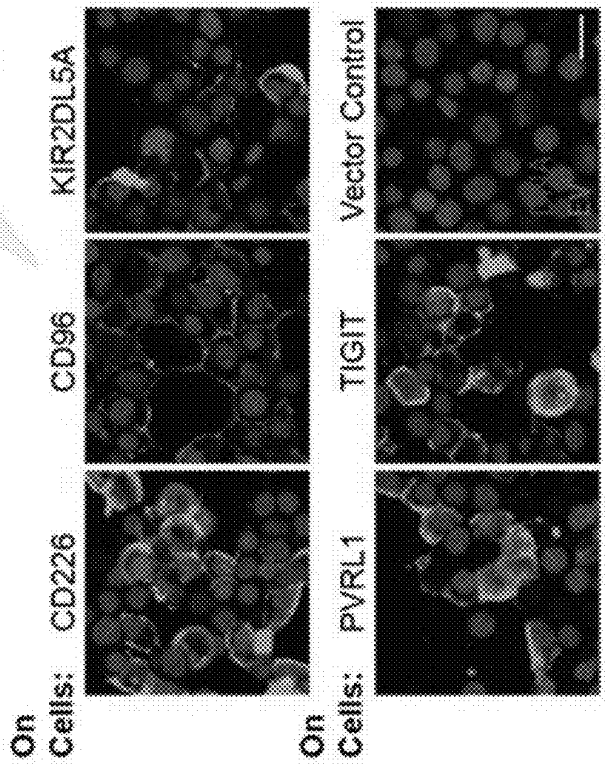

PVR Ligand
PVR
On Cells:
CD226
CD96
KIR2DL5A
On Cells:
PVRL1
TIGIT
Vector Control

FIG. 2F

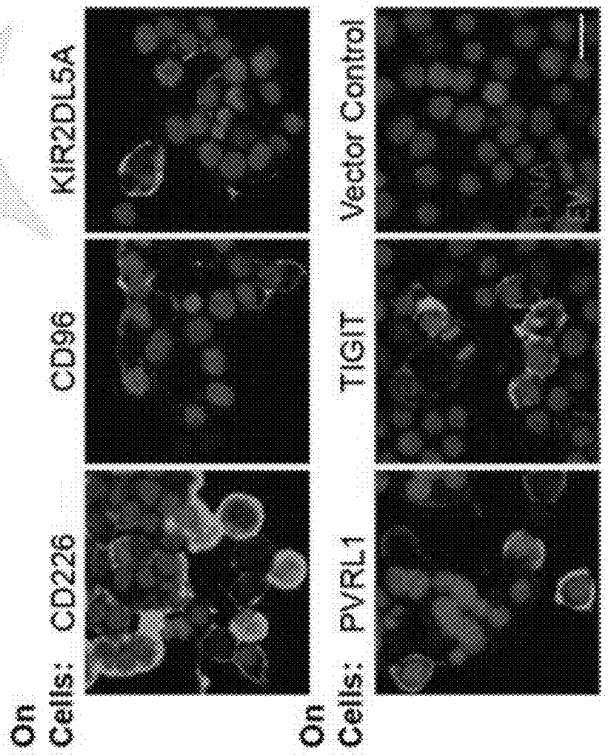

PVR Ligand
PVR
On Cells:
CD226
CD96
KIR2DL5A
On Cells:
PVRL1
TIGIT
Vector Control

FIG. 2G

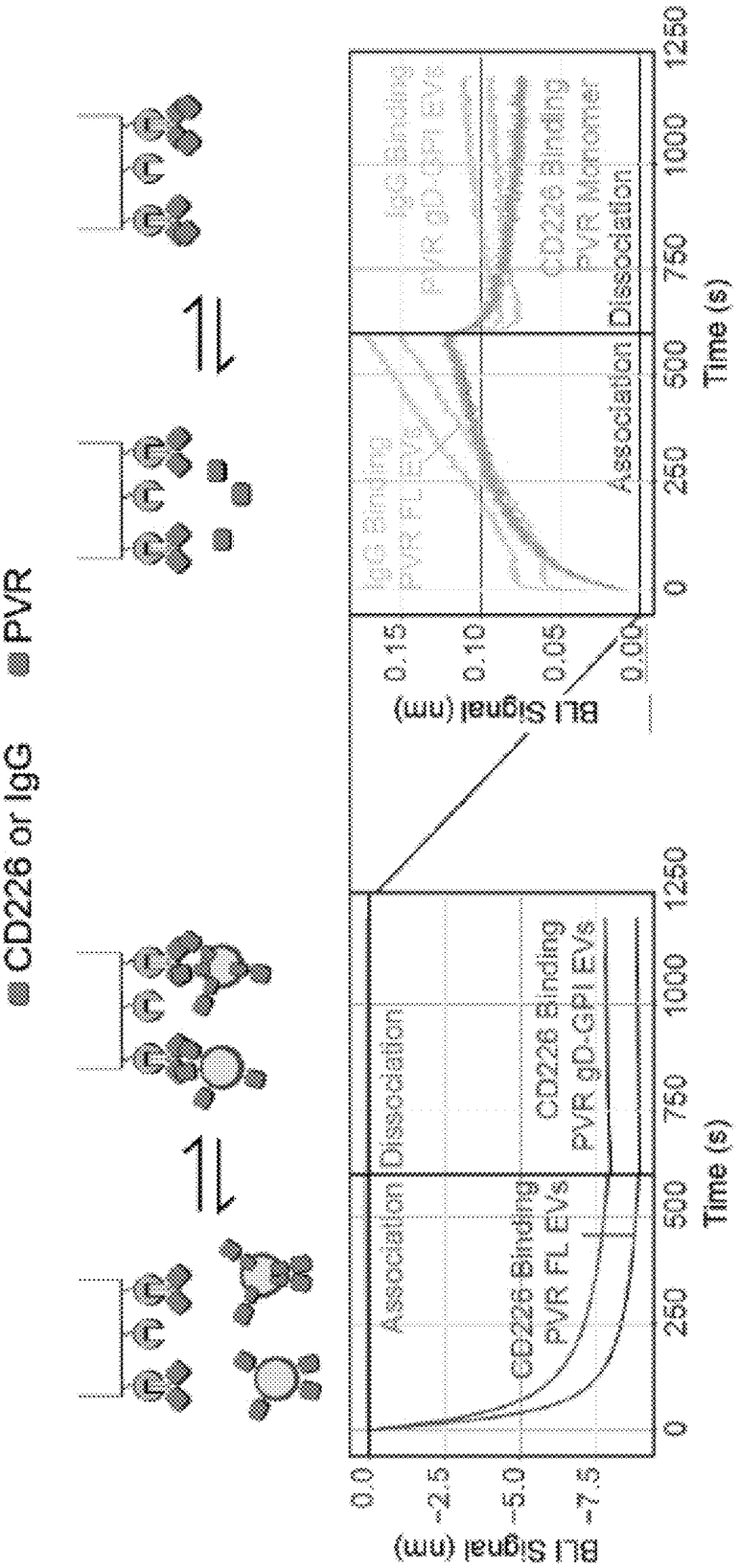

CD226 or IgG
PVR
Association
Dissociation
CD226 Binding
PVR FL EVs
CD226 Binding
PVR gD-GPI EVs
IgG Binding
PVR FL EVs
IgG Binding
PVR gD-GPI EVs
CD226 Binding
PVR Manner
BLI Signal (nm)
Time (s)
0.0
-2.5
-5.0
-7.5
0.15
0.10
0.05
0.00
0
250
500
750
1000
1250

FIG. 3A

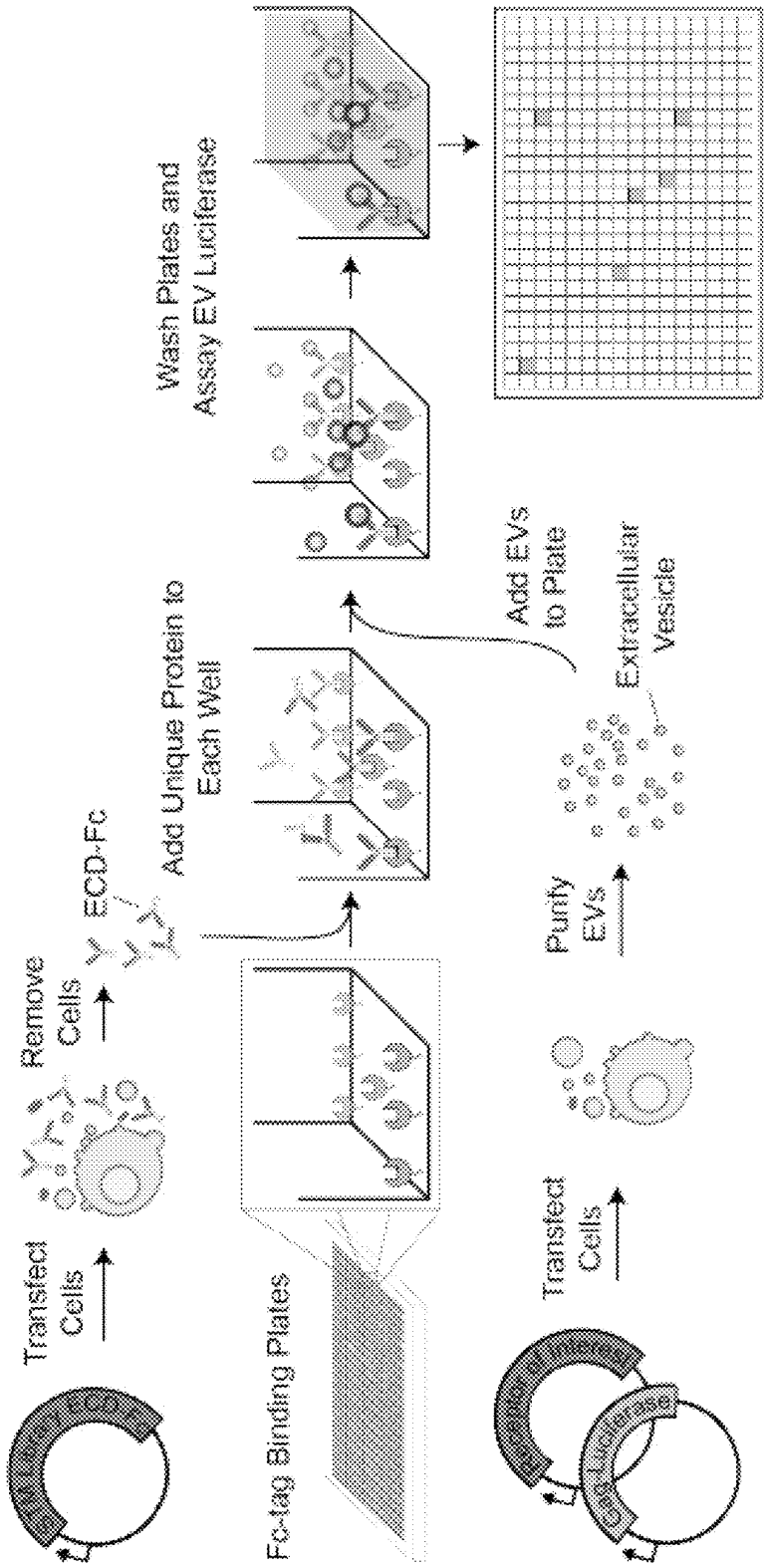

Transfect Cells
Remove Cells
ECD-Fc
Fc-tag Binding Plates
Add Unique Protein to Each Well
Add EVs to Plate
Wash Plates and Assay EV Luciferase
Transfect Cells
Purify EVs
Extracellular Vesicle

FIG. 3B

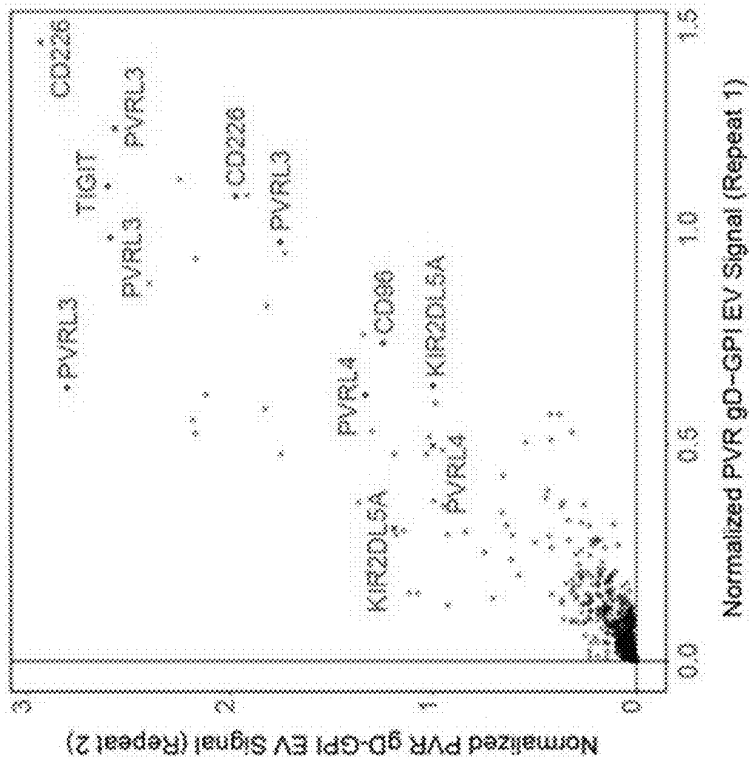

Normalized PVR gD-GPI EV Signal (Repeat 2)
Normalized PVR gD-GPI EV Signal (Repeat 1)
0.0
0.5
1.0
1.5
0
1
2
3
PVRL3
CD226
TIGIT
PVRL3
PVRL3
CD226
PVRL3
PVRL4
CD96
KIR2DL5A
PVRL4
KIR2DL5A

FIG. 3C

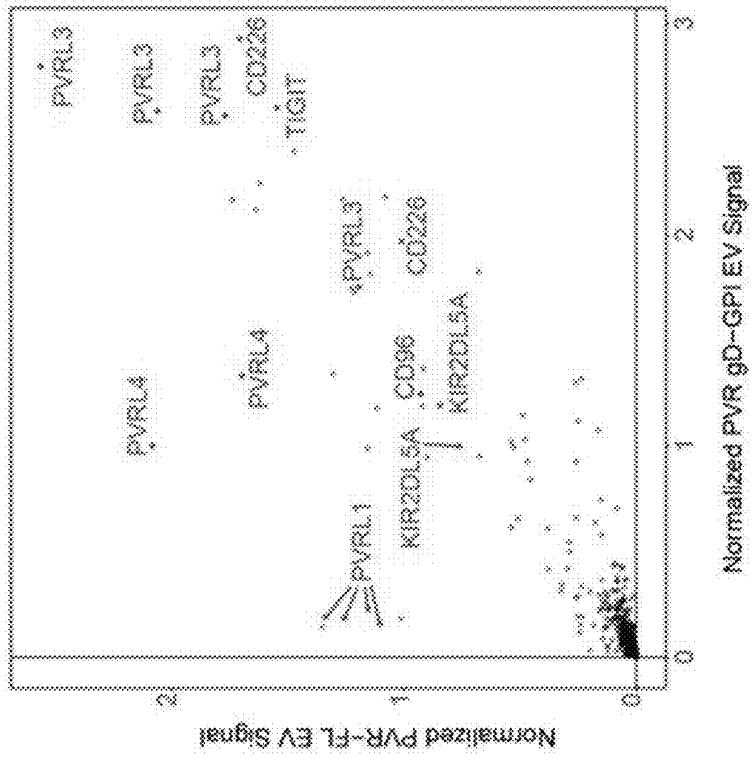

Normalized PVR-FL EV Signal
Normalized PVR gD-GPI EV Signal
0
1
2
3
PVRL3
PVRL3
PVRL3
CD226
TIGIT
PVRL4
PVRL4
PVRL3
PVRL1
KIR2DL5A
CD96
CD226
KIR2DL5A

FIG. 4A

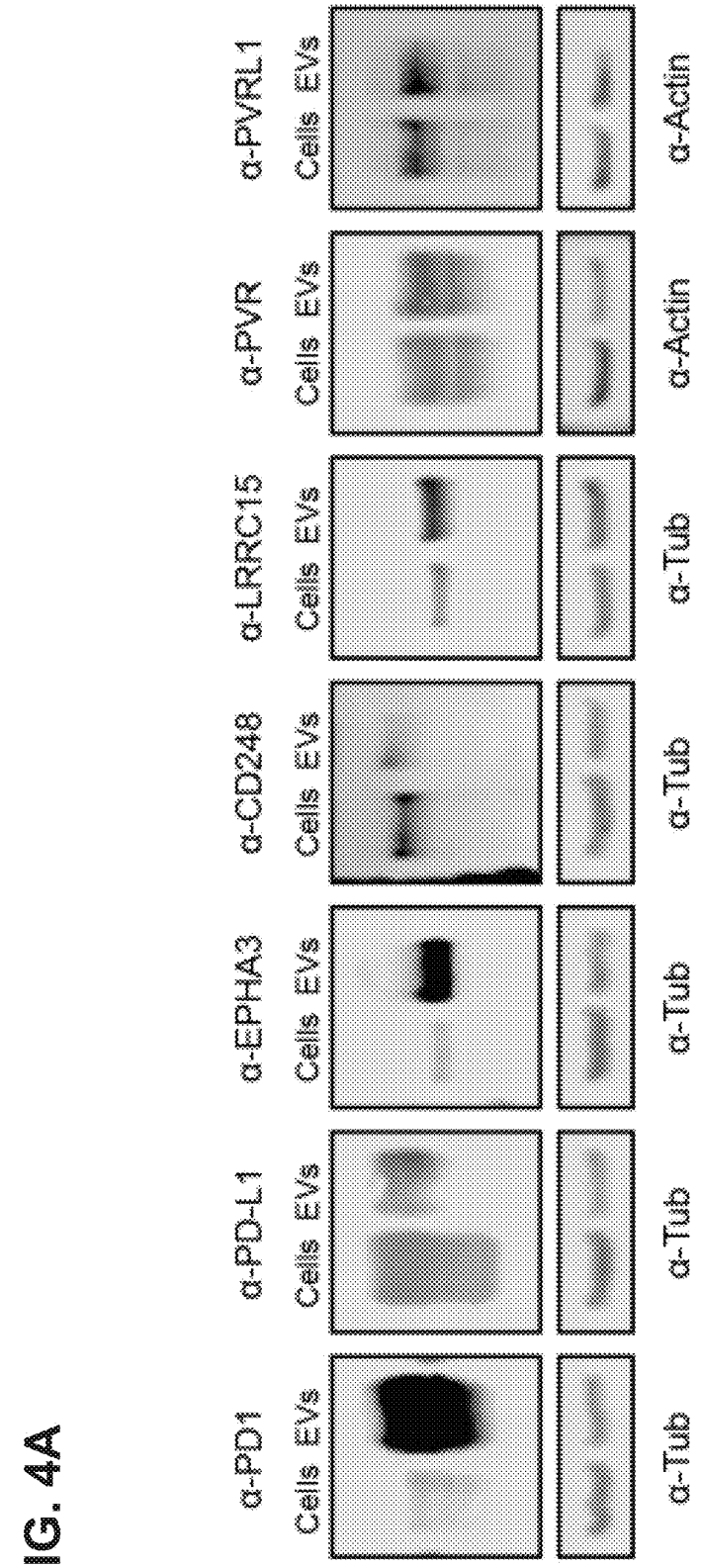

α-PD1
Cells EVs
α-Tub
α-PD-L1
Cells EVs
α-Tub
α-EPHA3
Cells EVs
α-Tub
α-CD248
Cells EVs
α-Tub
α-LRRC15
Cells EVs
α-Tub
α-PVR
Cells EVs
α-Actin
α-PVRL1
Cells EVs
α-Actin

FIG. 4C

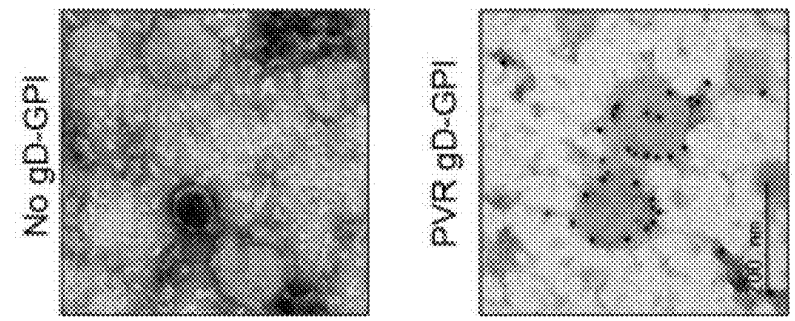
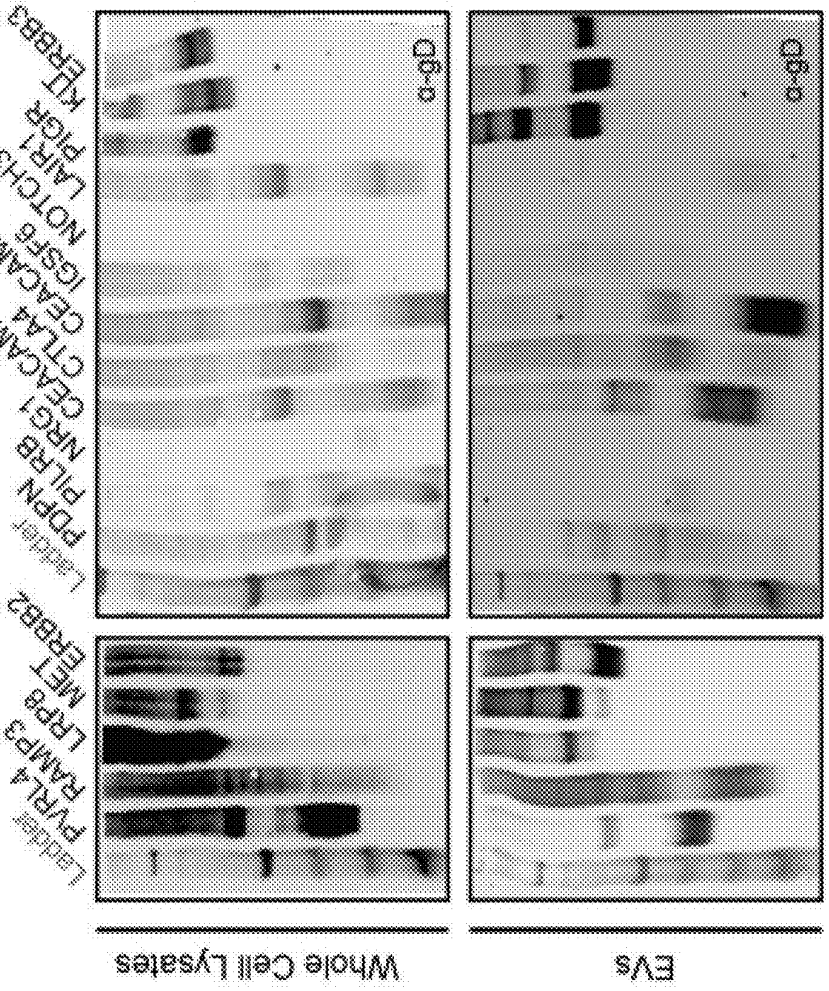

No gD-GPI
PVR gD-GPI

FIG. 4B


Whole Cell Lysates
EVs
α-gD
α-gD
Ladder
PVRL4
RAMP3
LRP8
MET
ERBB2
Ladder
FDPN
PLRB
NRG1
CEACAM
CTLA4
CEACAM3
IGSF6
NOTCH3
LAIR1
PIGR
KLERBB3

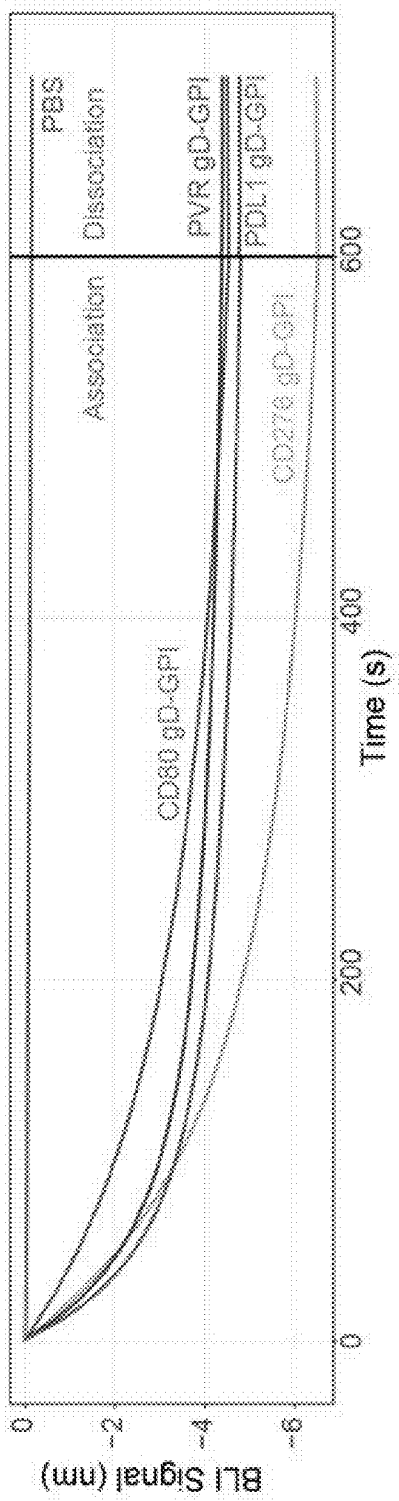

PBS
Dissociation
Association
PVR gD-GPI
PDL1 gD-GPI
CD80 gD-GPI
CD270 gD-GPI
BLI Signal (nm)
0
-2
-4
-6
0
200
400
600
Time (s)

FIG. 4D

FIG. 5A

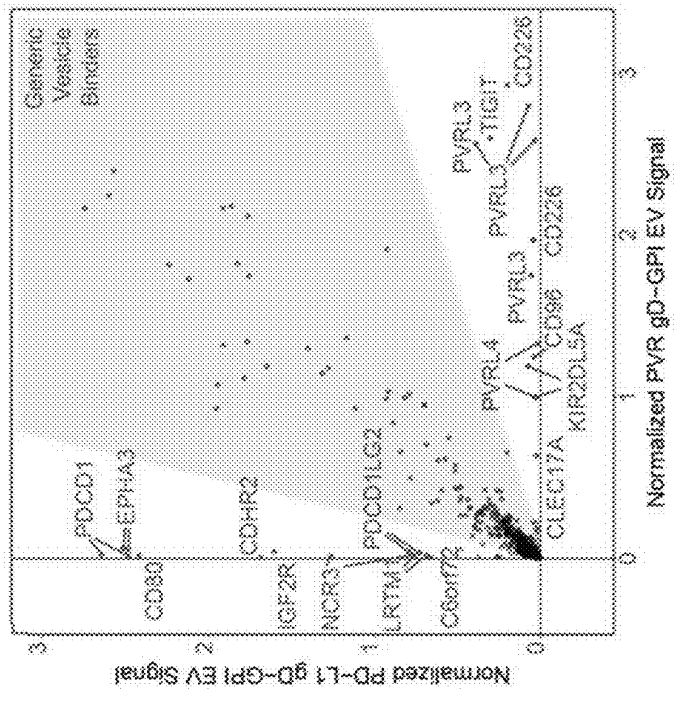

Normalized PD-L1 gD-GPI EV Signal
Normalized PVR gD-GPI EV Signal
Generic
Vesicle
Binders
PDCD1
EPHA3
CD80
CDHR2
IGF2R
NCR3
PDCD1LG2
LRTM1
CD300LF
PVRL4
PVRL3
CD96
CLEC17A
KIR2DL5A
PVRL3
TIGIT
CD226
PVRL3
CD226

FIG. 5B

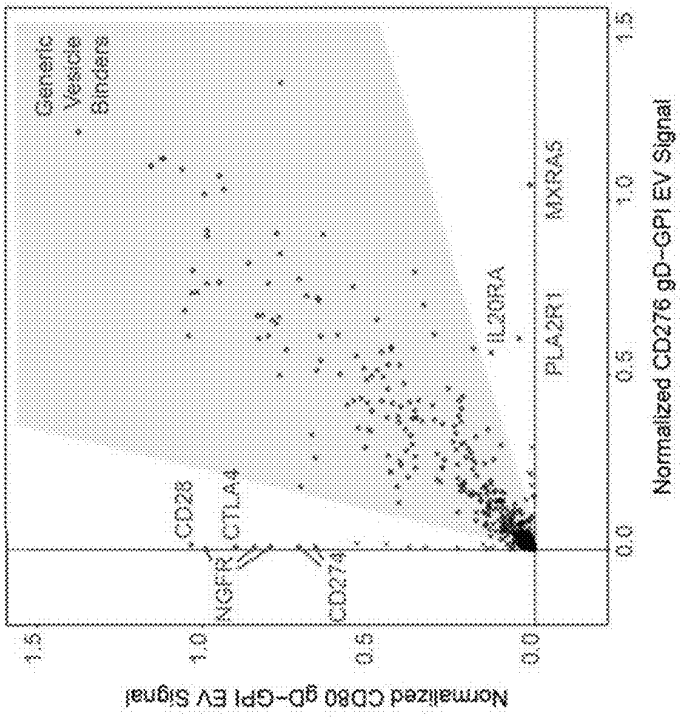

Normalized CD80 gD-GPI EV Signal
Normalized CD276 gD-GPI EV Signal
Generic
Vesicle
Binders
CD28
CTLA4
NGFR
CD274
IL20RA
PLA2R1
MXRA5

FIG. 5C

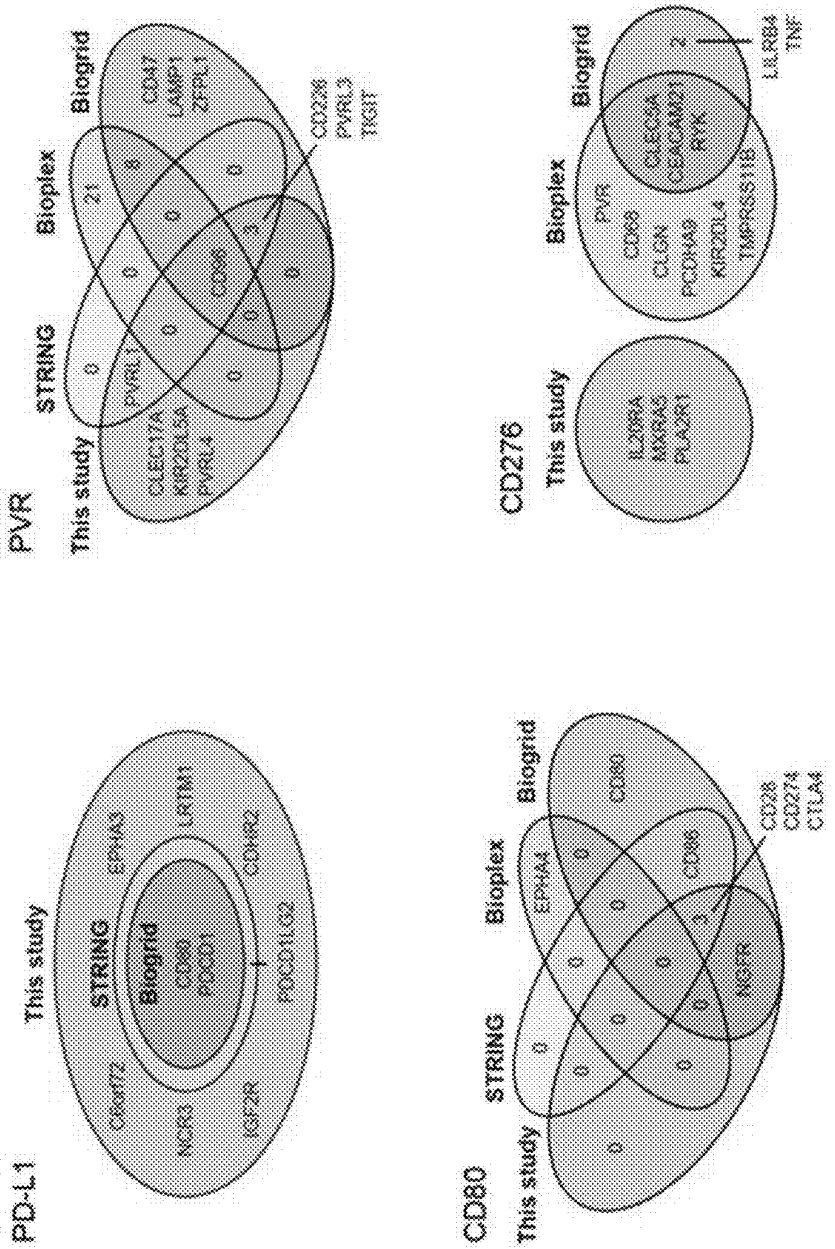

PVR
This study
STRING
Bioplex
Biogrid
CLEC7A
KIR2DL4
PVRL4
PVRL2
CD96
CD47
LAIR1
ZFPL1
CD226
PVRL3
TIGIT
CD276
This study
Bioplex
Biogrid
IL23RA
MKRN3
PLA2R1
PVR
CD69
CLGN
PCDHA9
KIR2DL4
TMPRSS11B
CLEC5A
CEACAM21
RYK
LILRB4
TNF
PD-L1
This study
STRING
Biogrid
CD247
NCR3
IGF2R
EPHA3
LRTM1
CD96
PECR1
CD4R2
PDCD1LG2
CD80
This study
STRING
Bioplex
Biogrid
EPHA4
CD80
CD96
NCR3R
CD28
CD274
CTLA4

FIG. 6A

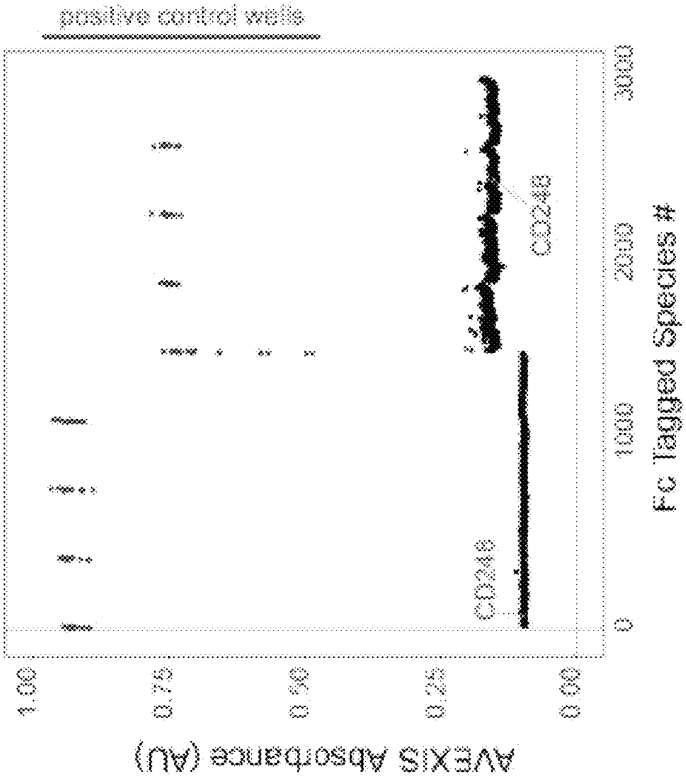

positive control wells
AVEXIS Absorbance (AU)
1.00
0.75
0.50
0.25
0.00
0
1000
2000
3000
Fc Tagged Species #
CD248
CD248

FIG. 6B

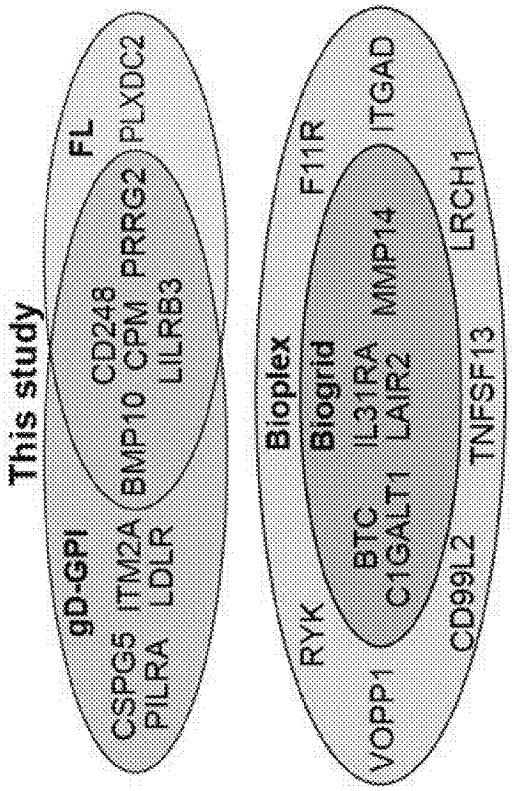

This study
FL
gD-GPI
PLXDC2
CD248
PRRG2
BMP10
CPM
LILRB3
ITM2A
LDLR
CSPG5
PILRA
Bioplex
Biogrid
F11R
ITGAD
LRCH1
MMP14
IL31RA
LAIR2
BTC
C1GALT1
TNFSF13
CD99L2
RYK
VOPP1

FIG. 7A

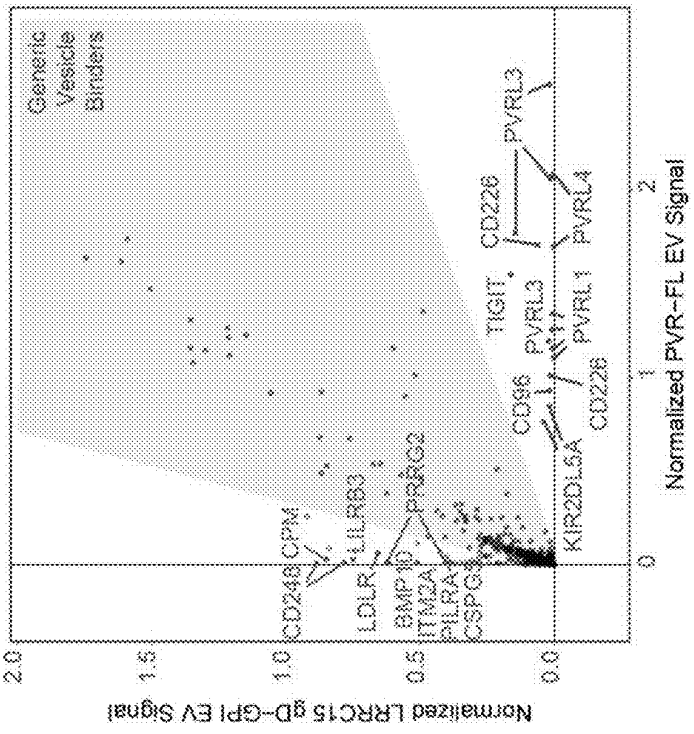

Normalized LRRC15 gD-GPI EV Signal
2.0
1.5
1.0
0.5
0.0
Generic
Vesicle
Binders
CPM
CD248
LILRB3
LDLR
BMP1
ITM2A
PILRA
CSPG4
PRRG2
TIGIT
CD226
PVRL3
CD96
PVRL3
PVRL1
PVRL4
CD226
KIR2DL5A
0
1
2
Normalized PVR-FL EV Signal

FIG. 7B

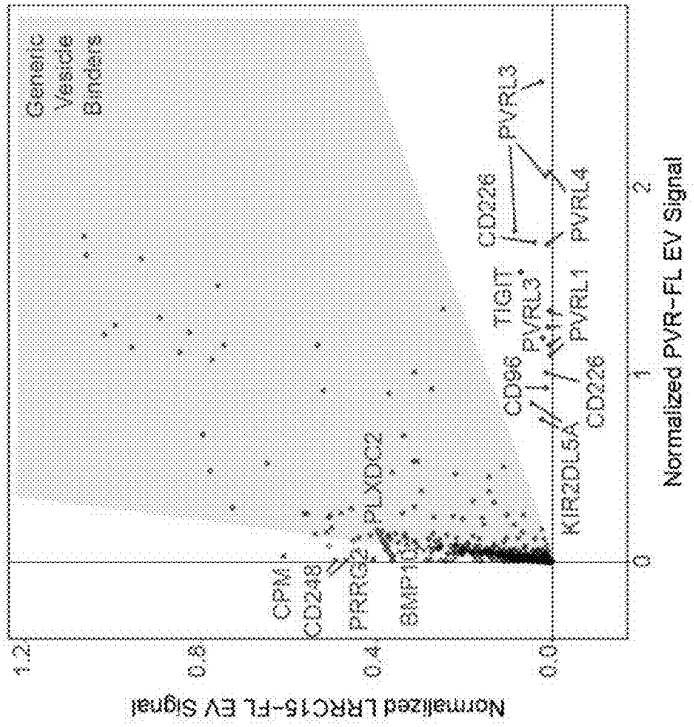

Normalized LRRC15-FL EV Signal
1.2
0.8
0.4
0.0
Generic
Vesicle
Binders
CPM
CD248
PRRG2
PLXDC2
BMP10
TIGIT
CD226
PVRL3
CD96
PVRL3
PVRL1
PVRL4
CD226
KIR2DL5A
0
1
2
Normalized PVR-FL EV Signal

FIG. 8A

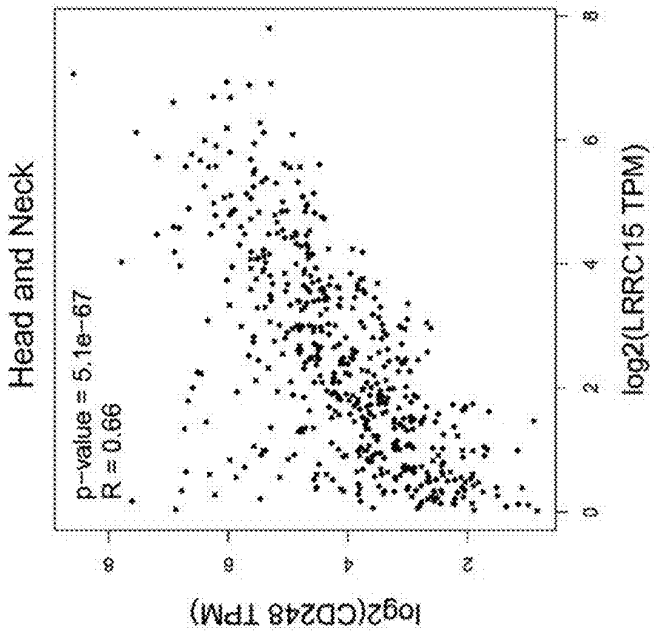
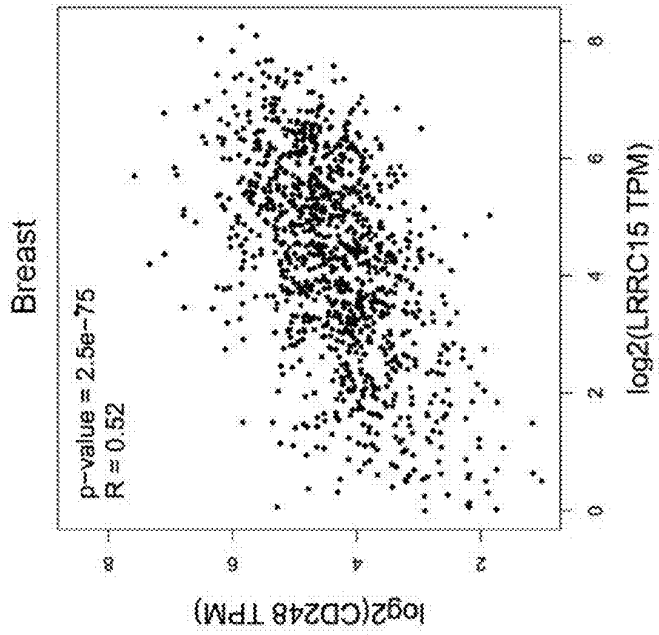
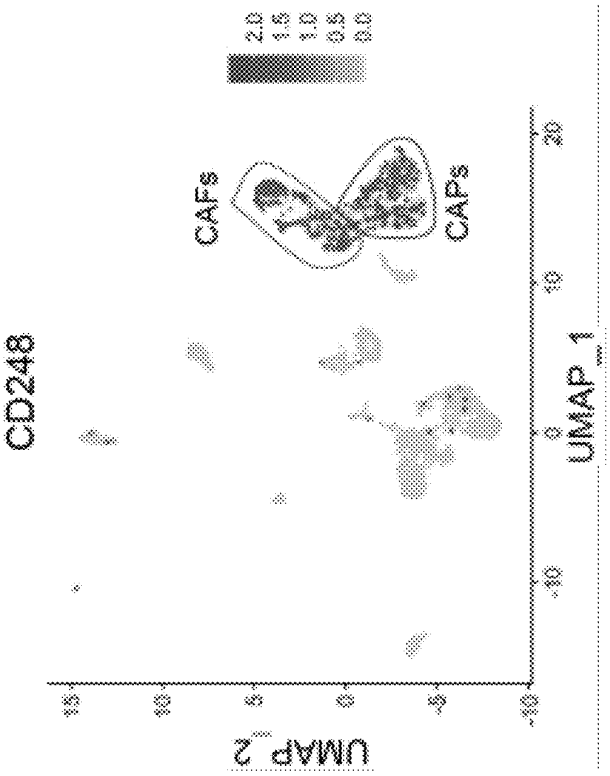

Head and Neck
p−value = 5.1e−67
R = 0.66
log2(CD248 TPM)
log2(LRRC15 TPM)
0
2
4
6
8

FIG. 8B


Breast
p−value = 2.5e−75
R = 0.52
log2(CD248 TPM)
log2(LRRC15 TPM)
0
2
4
6
8


CD248
CAFs
CAPs
UMAP_1
UMAP_2
2.0
1.5
1.0
0.5
0.0

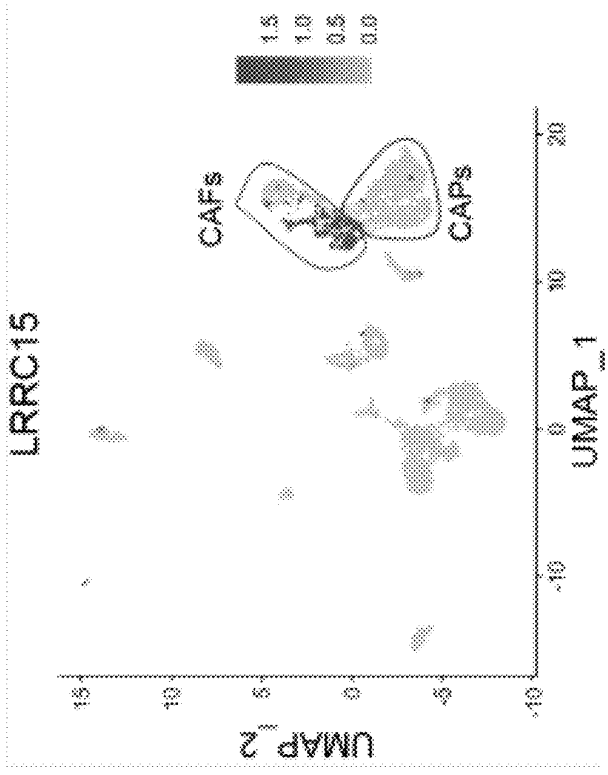

LRRC15
CAFs
CAPs
UMAP_1
UMAP_2
1.5
1.0
0.5
0.0

FIG. 8C

FIG. 9A

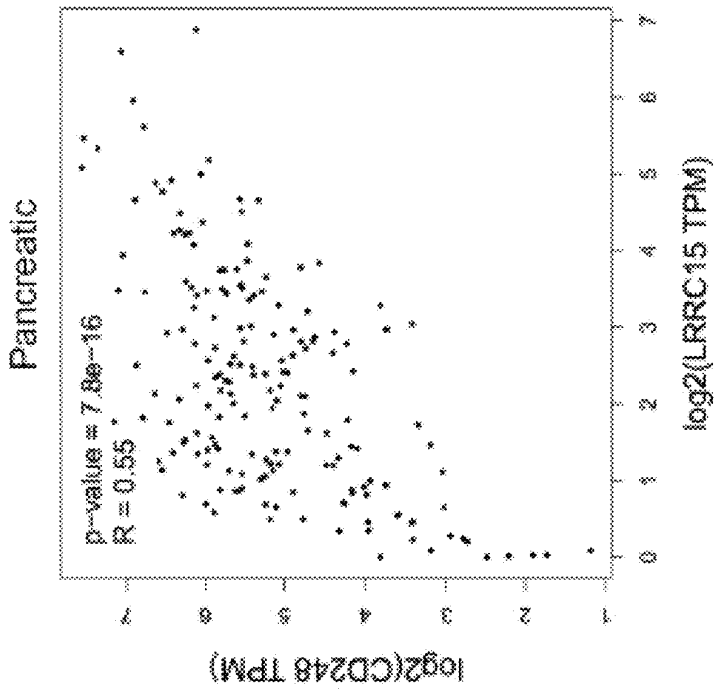
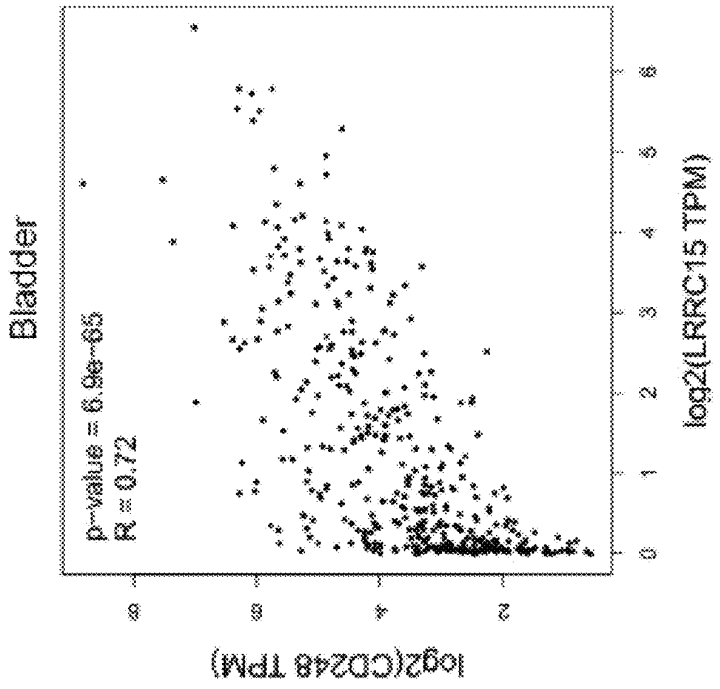
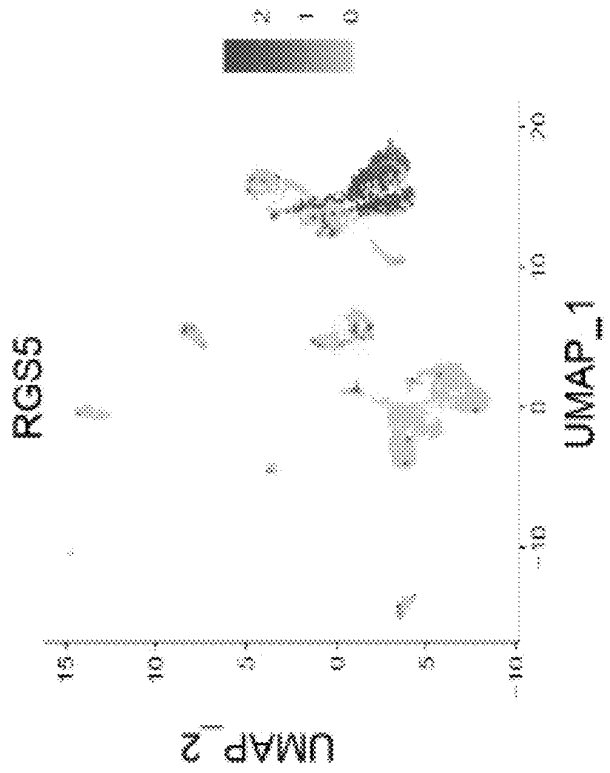

Pancreatic
p-value = 7.8e-16
R = 0.55
log2(CD248 TPM)
log2(LRRC15 TPM)

FIG. 9B


Bladder
p-value = 6.9e-65
R = 0.72
log2(CD248 TPM)
log2(LRRC15 TPM)


RGS5
UMAP_2
UMAP_1
2
1
0
15
10
5
0
-5
-10
-10
0
10
20

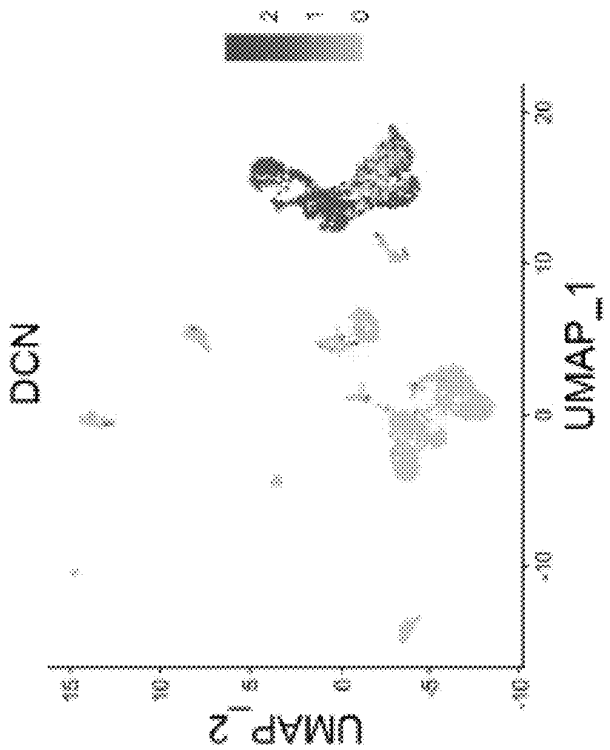

DCN
UMAP_2
UMAP_1
2
1
0
15
10
5
0
-5
-10
-10
0
10
20

FIG. 9C

FIG. 10A

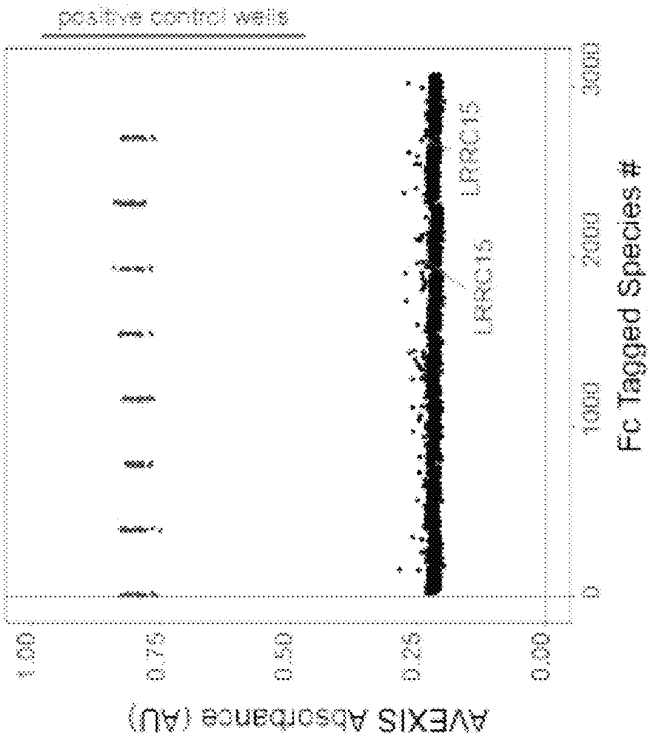


FIG. 10B

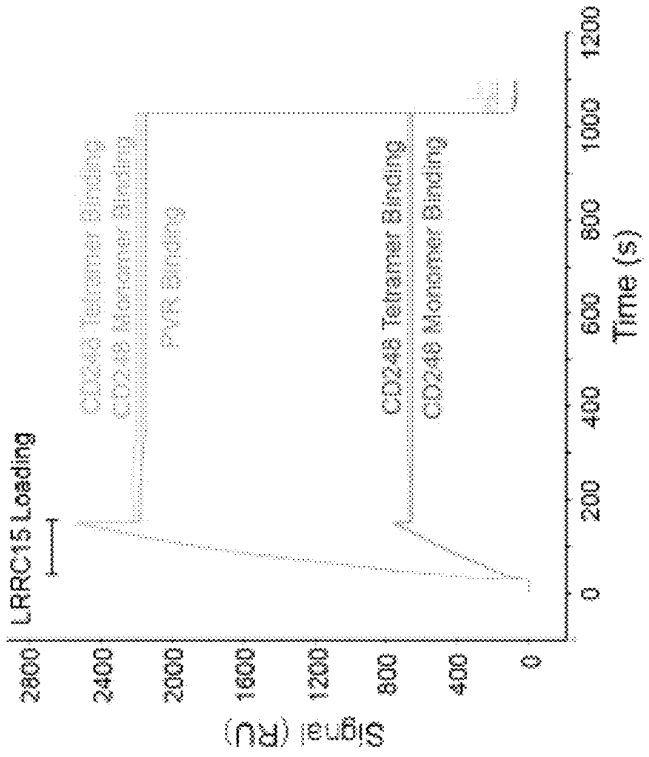


FIG. 11A

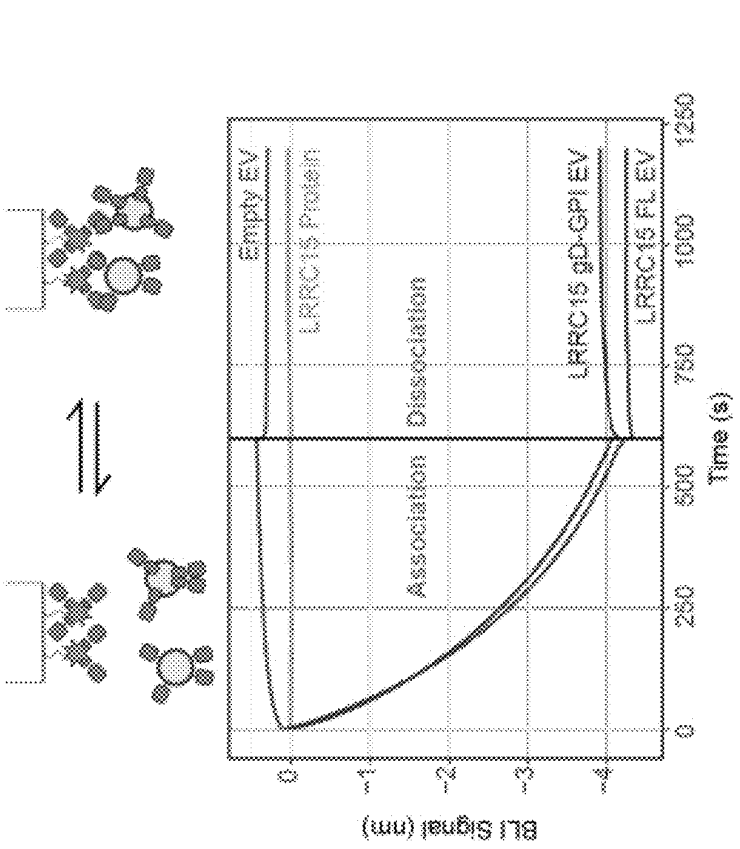

CD248
LRRC15
Empty EV
LRRC15 Protein
LRRC15 gD-GPI EV
LRRC15 FL EV
Association
Dissociation
BLI Signal (nm)
Time (s)
0
-1
-2
-3
-4
0
250
500
750
1000
1250

FIG. 11B

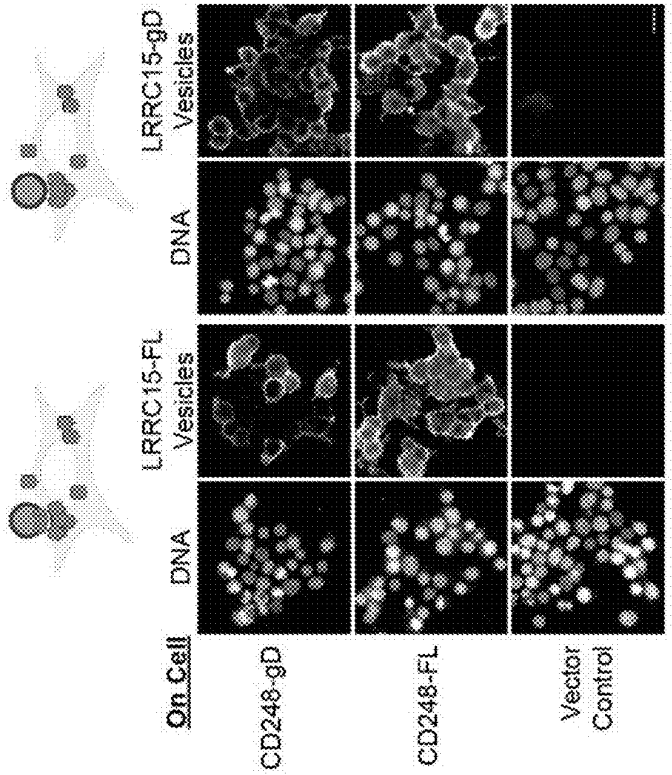


FIG. 11C

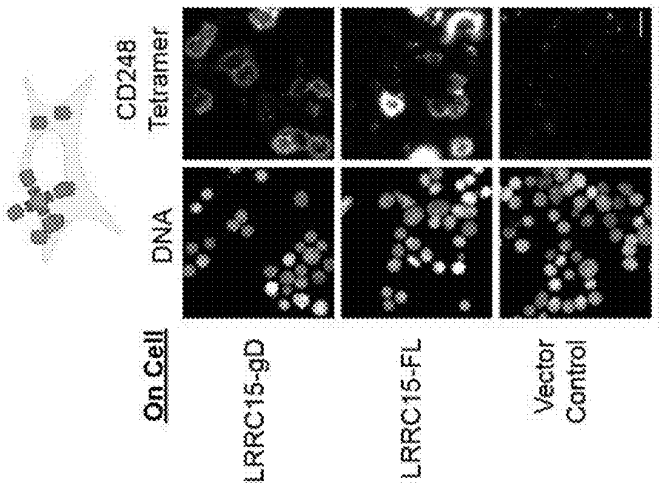
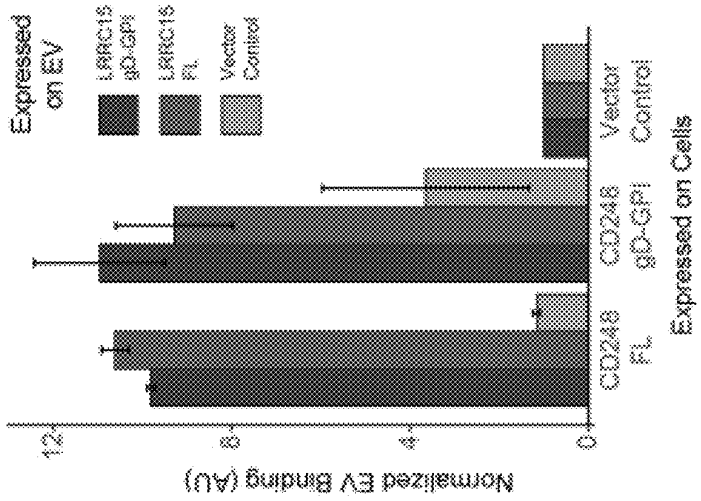


FIG. 11D


Expressed on EV
LRRC15 gD-GPI
LRRC15 FL
Vector Control
Normalized EV Binding (AU)
12
8
4
0
CD248 FL
CD248 gD-GPI
Vector Control
Expressed on Cells

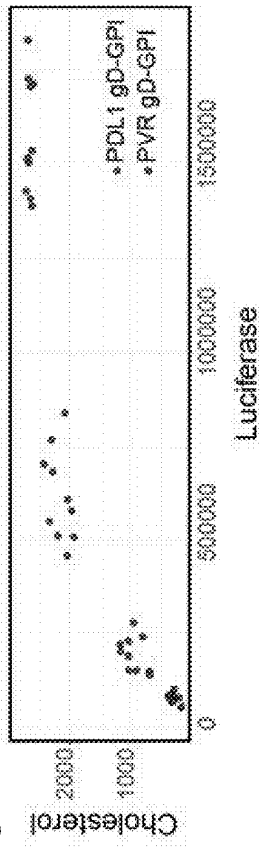


FIG. 12A

FIG. 12B

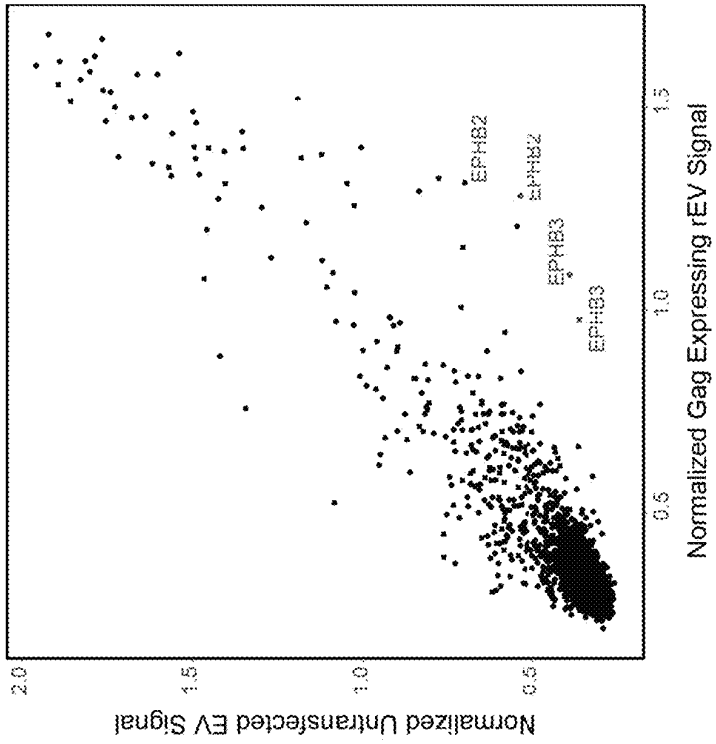

EPHB2
EPHB2
EPHB3
EPHB3
0.5
1.0
1.5
Normalized Gag Expressing rEV Signal
2.0
1.5
1.0
0.5
Normalized Untransfected EV Signal

FIG. 12C

FIG. 13B

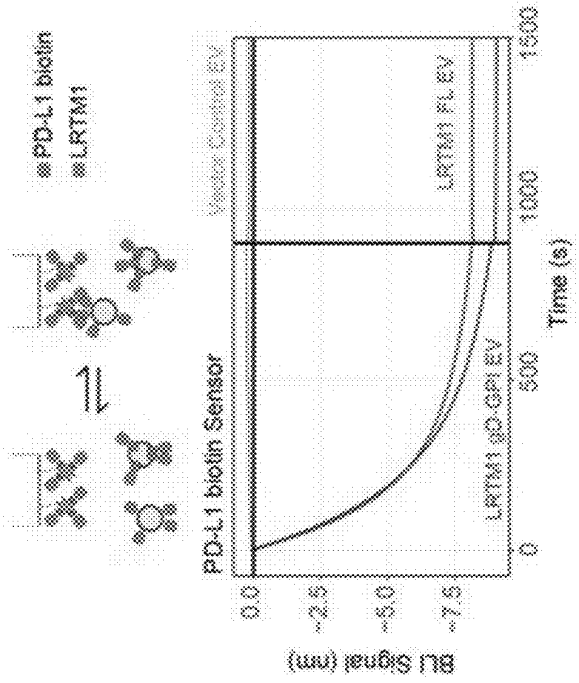


FIG. 13A

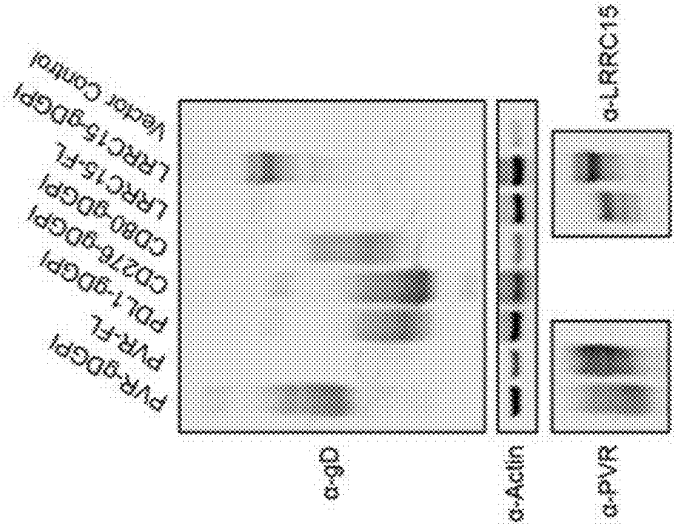


FIG. 13C

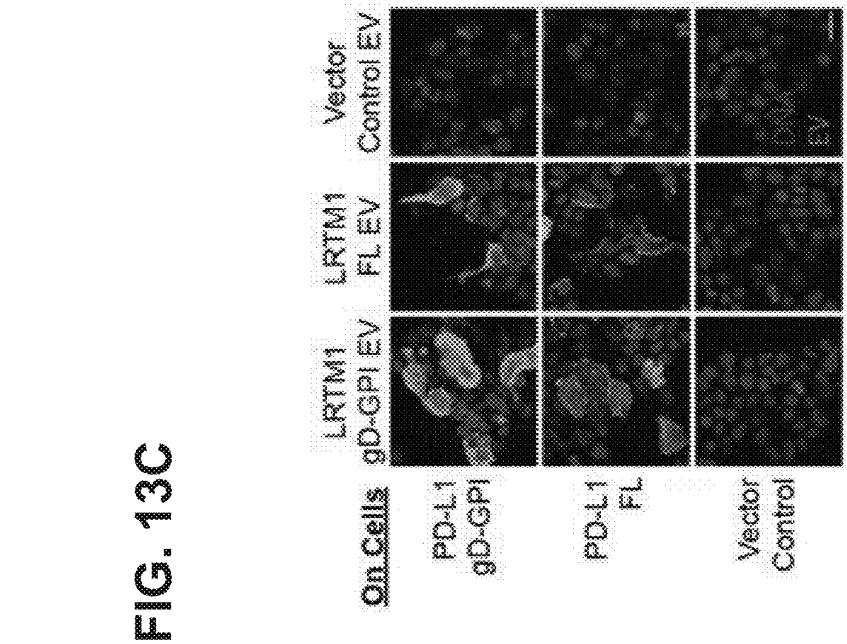


FIG. 13D

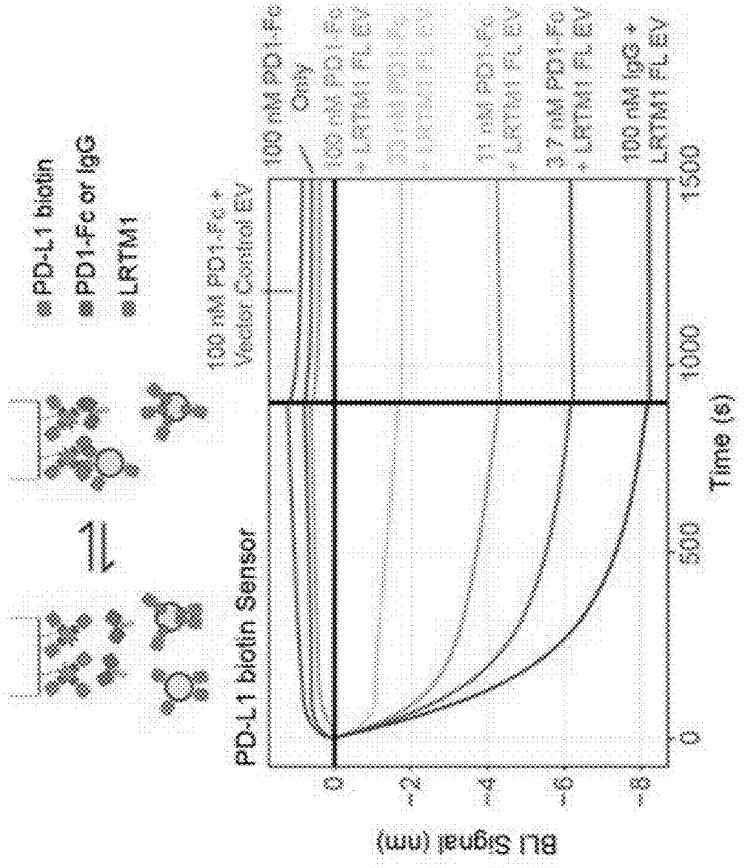


FIG. 14A

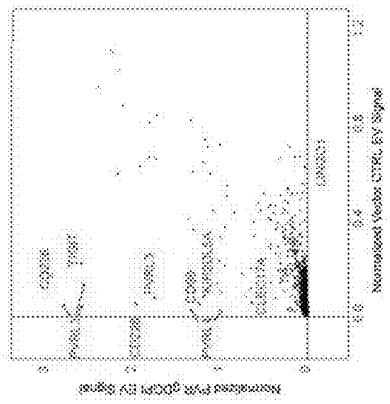


FIG. 14B

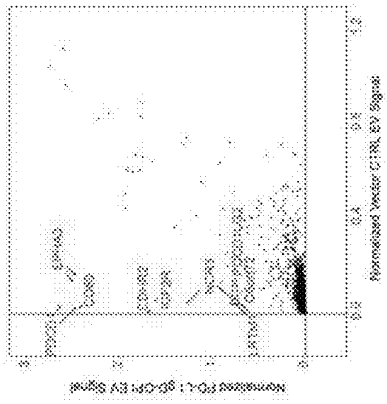


FIG. 14C

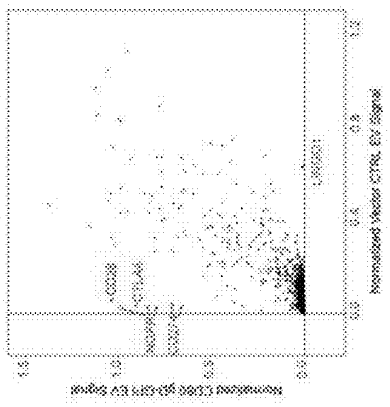


FIG. 14D

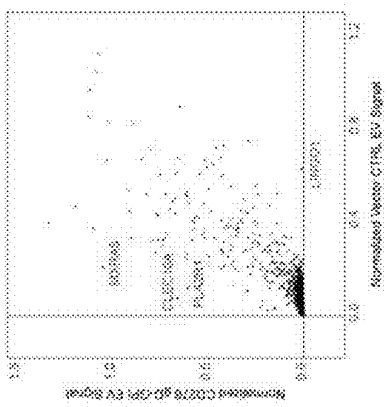


FIG. 14E

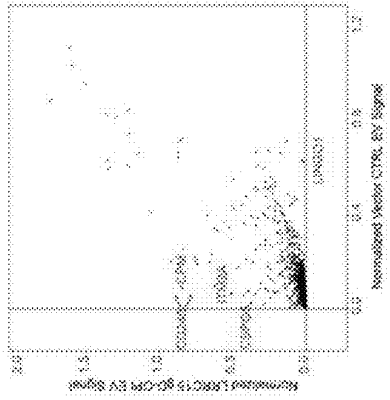
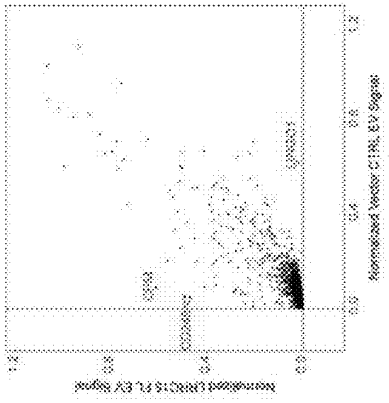


FIG. 14F



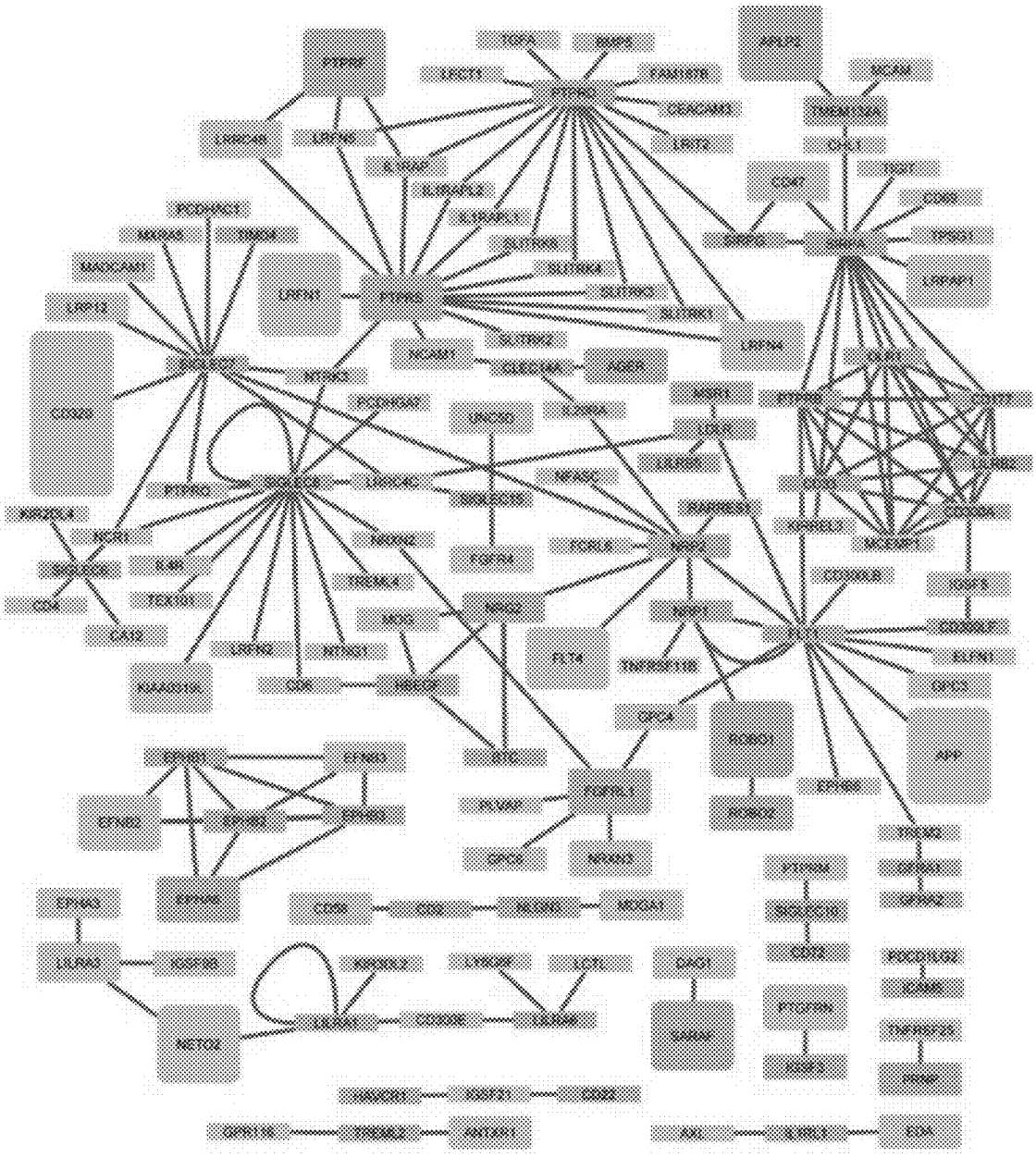


FIG. 15

FIG. 16A

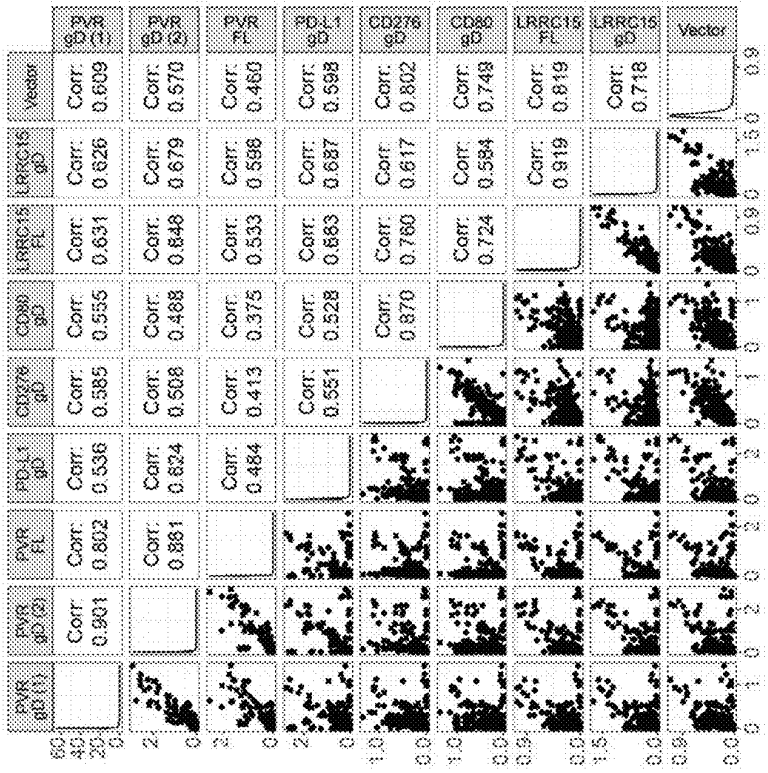
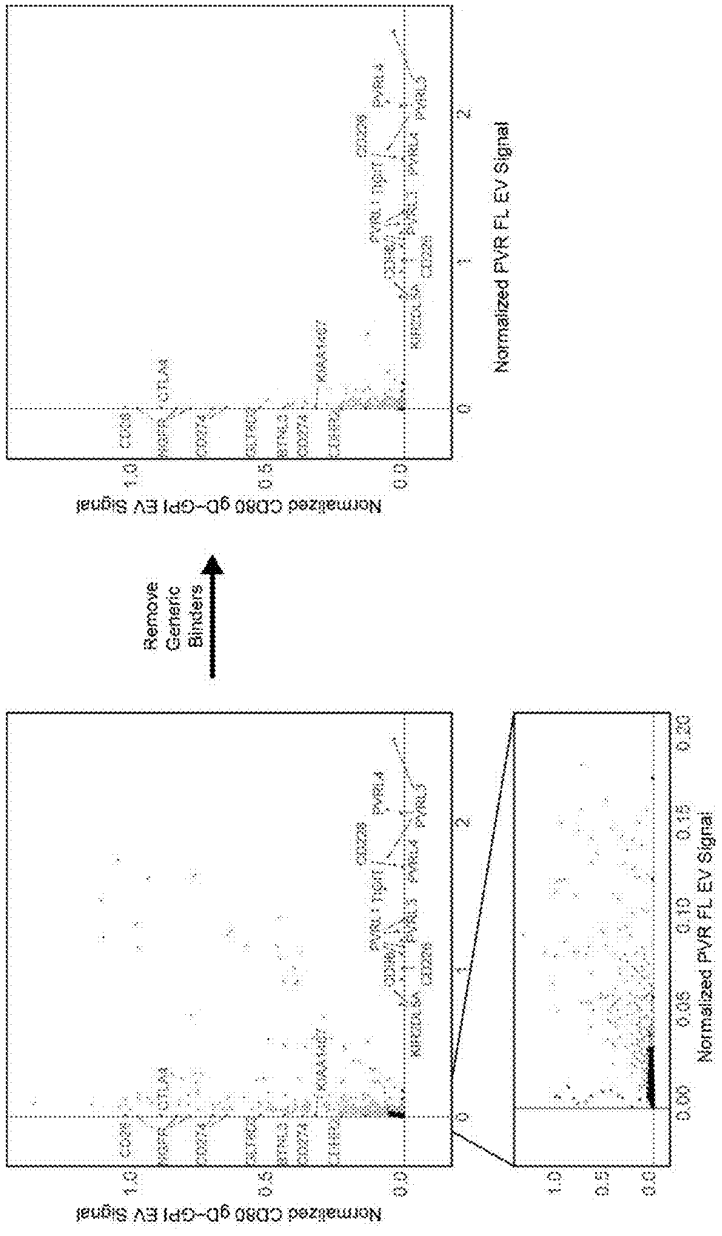

PVR gD (1)
PVR gD (2)
PVR FL
PDL1 gD
CD276 gD
CD80 gD
LRRC15 FL
LRRC15 gD
Vector
gD PVR (1)
gD PVR (2)
PVR FL
PDL1 gD
CD276 gD
CD80 gD
LRRC15 FL
LRRC15 gD
Vector
Corr. 0.609
Corr. 0.570
Corr. 0.460
Corr. 0.598
Corr. 0.802
Corr. 0.748
Corr. 0.819
Corr. 0.718
Corr. 0.626
Corr. 0.679
Corr. 0.598
Corr. 0.687
Corr. 0.617
Corr. 0.584
Corr. 0.919
Corr. 0.631
Corr. 0.648
Corr. 0.533
Corr. 0.663
Corr. 0.760
Corr. 0.724
Corr. 0.555
Corr. 0.488
Corr. 0.375
Corr. 0.528
Corr. 0.870
Corr. 0.585
Corr. 0.508
Corr. 0.413
Corr. 0.551
Corr. 0.536
Corr. 0.624
Corr. 0.484
Corr. 0.802
Corr. 0.881
Corr. 0.901

FIG. 16B


Normalized CD80 gD-GPI EV Signal
Normalized PVR FL EV Signal
Remove Generic Binders
Normalized CD80 gD-GPI EV Signal
Normalized PVR FL EV Signal

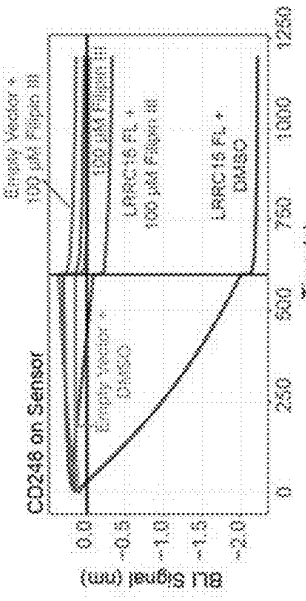


FIG. 17B

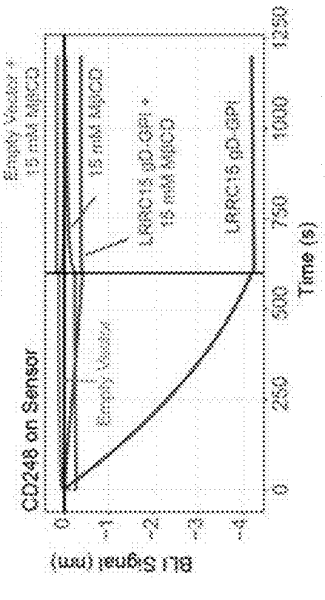


FIG. 17D

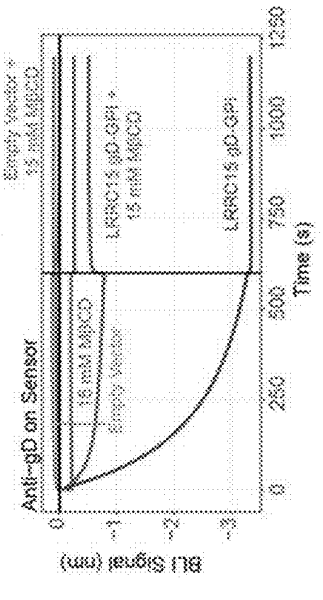


FIG. 17F

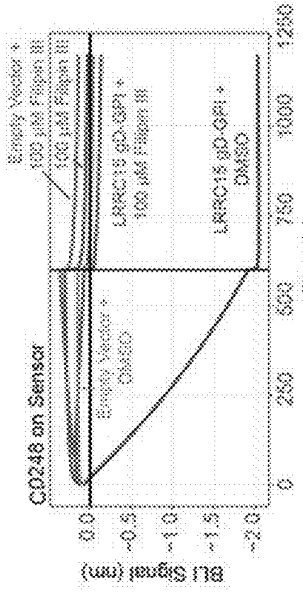


FIG. 17A

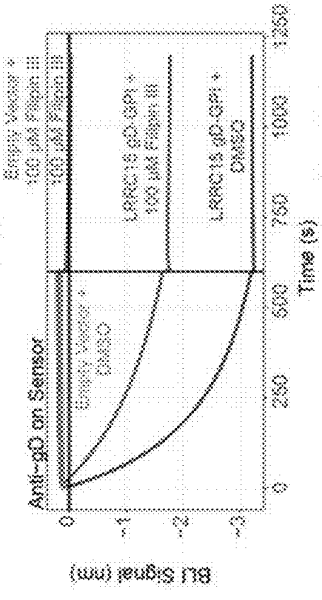


FIG. 17C

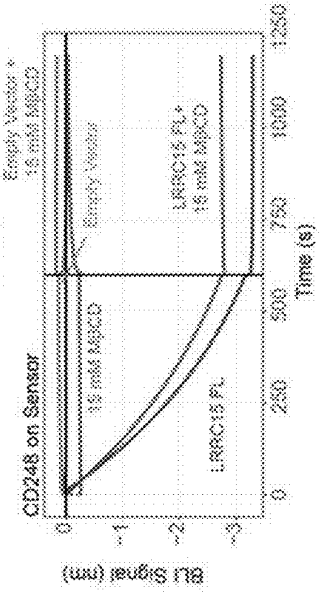


FIG. 17E

FIG. 18A

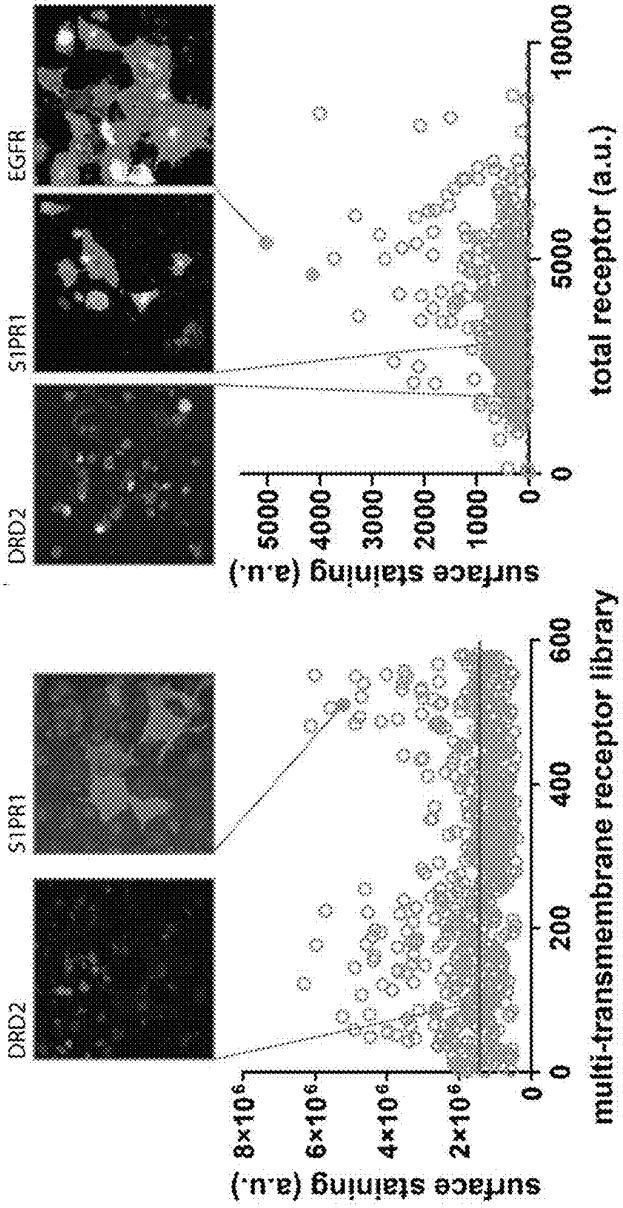


FIG. 18B

FIG. 18C

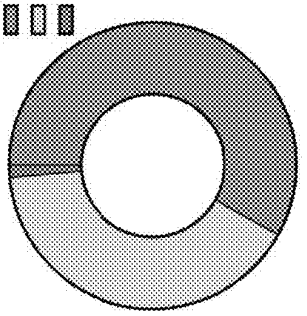


FIG. 18D

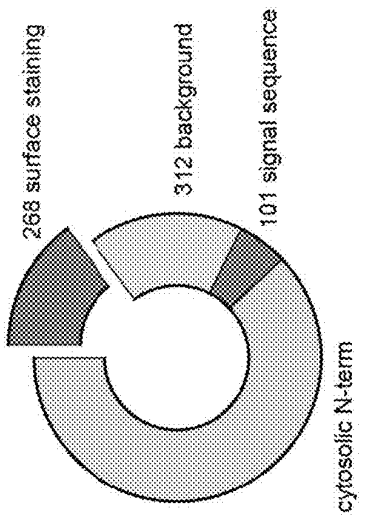


FIG. 19A

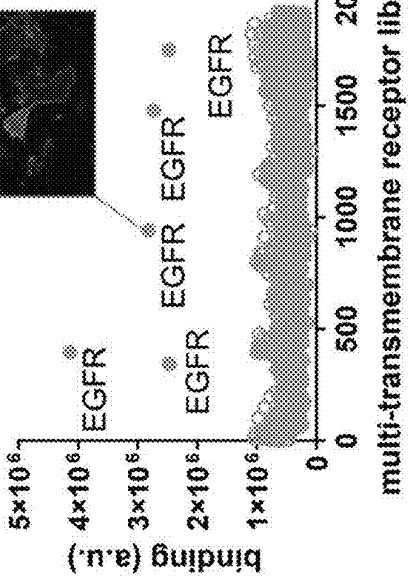


FIG. 19B

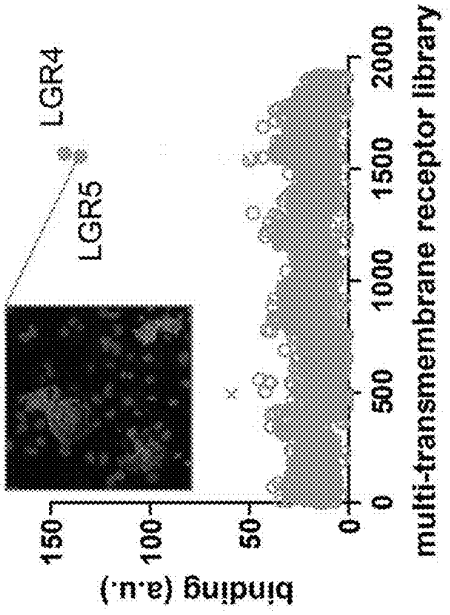


FIG. 19C

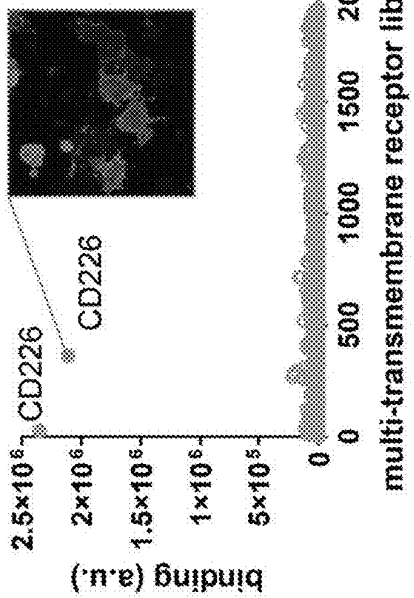


FIG. 19D

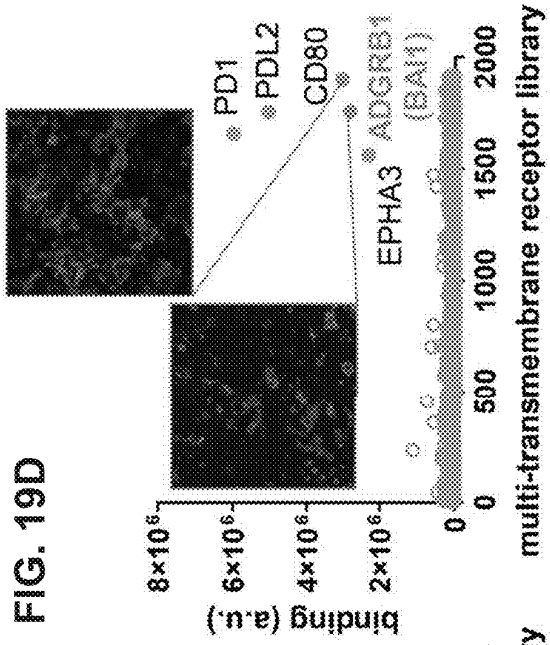


FIG. 20A

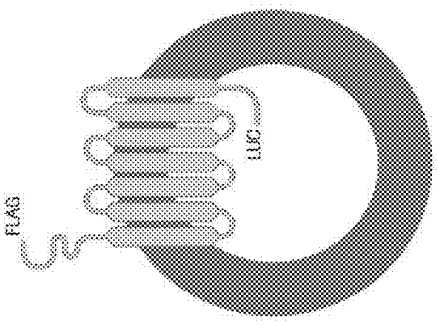


FIG. 20B

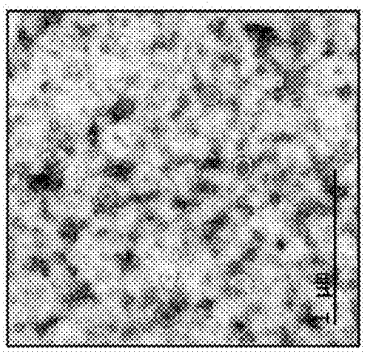


FIG. 20C

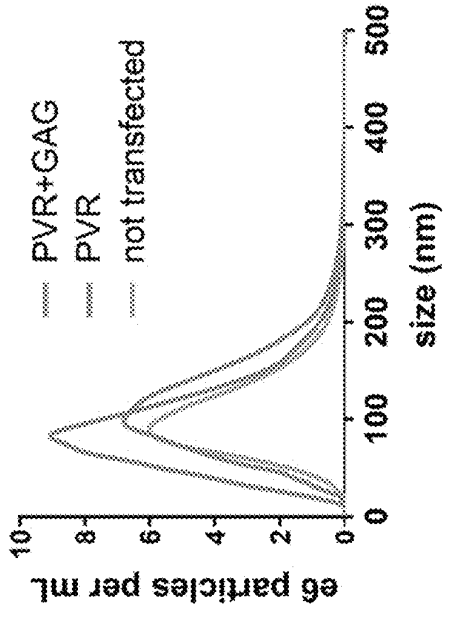
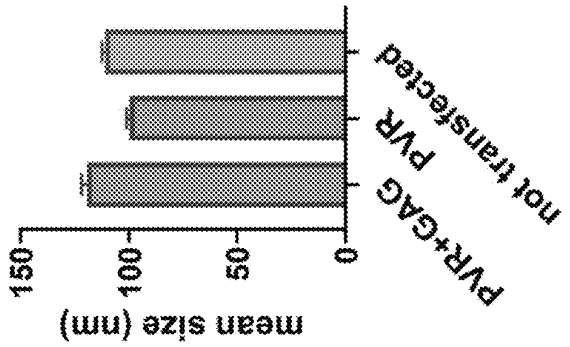


FIG. 20D



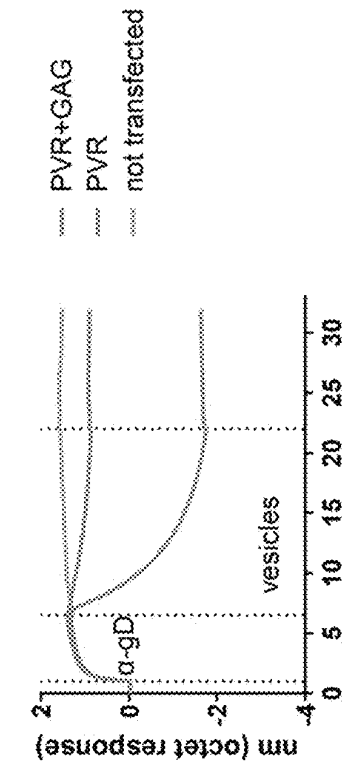


FIG. 20E

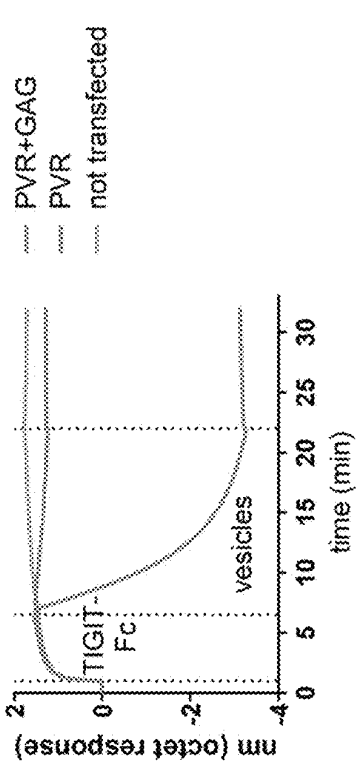


FIG. 20F

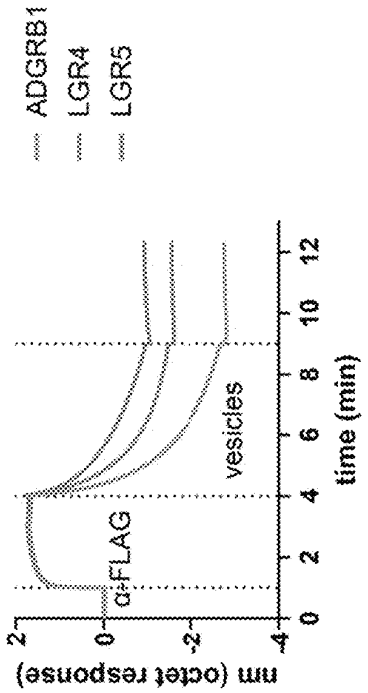


FIG. 20G

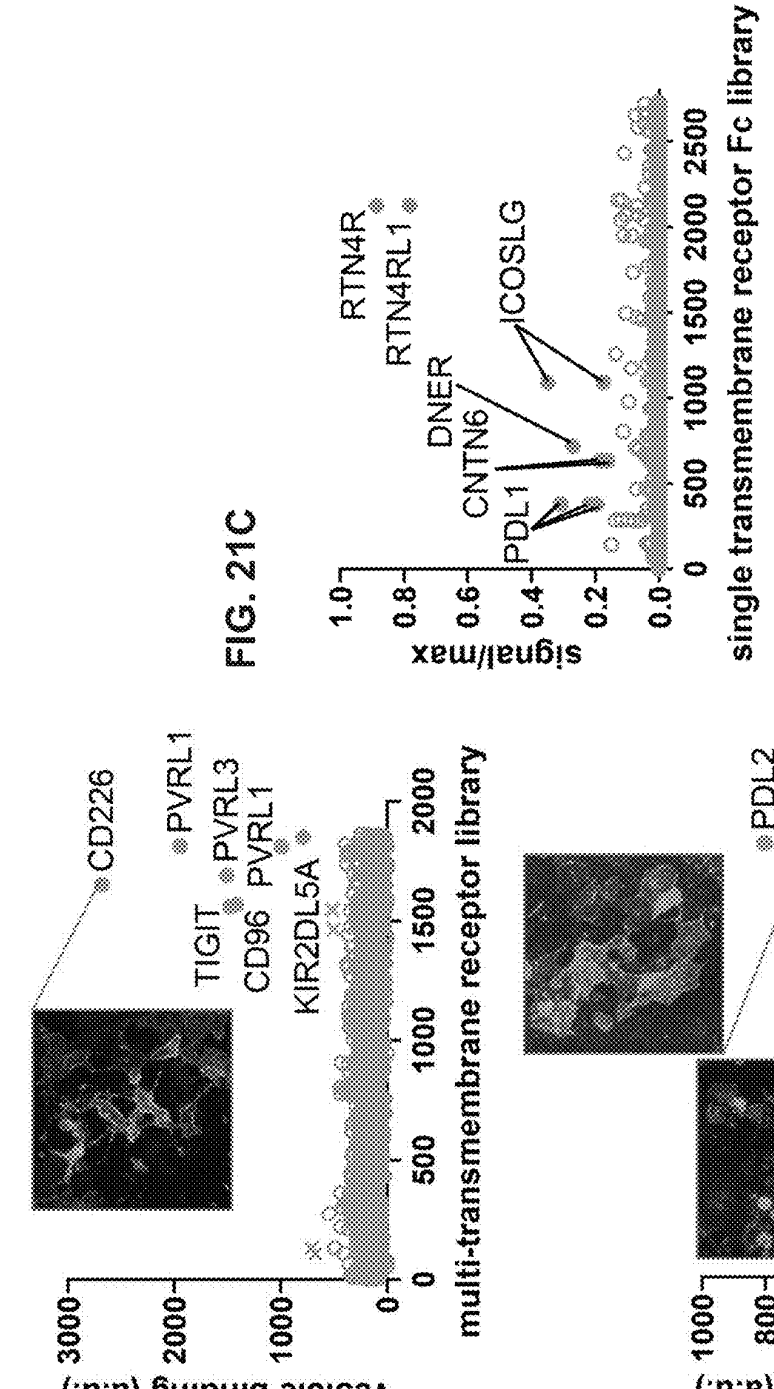


FIG. 21A
vesicle binding (a.u.)
3000
2000
1000
0
0 500 1000 1500 2000
multi-transmembrane receptor library
CD226
PVRL1
TIGIT
PVRL3
CD96
PVRL1
KIR2DL5A
FIG. 21C
signal/max
1.0
0.8
0.6
0.4
0.2
0.0
0 500 1000 1500 2000 2500
single transmembrane receptor Fc library
RTN4R
RTN4RL1
DNER
CNTN6
PDL1
ICOSLG
FIG. 21B
vesicle binding (a.u.)
1000
800
600
400
200
0
0 500 1000 1500 2000
multi-transmembrane receptor library
PDL2
CD80
ADGRB1
PDL1

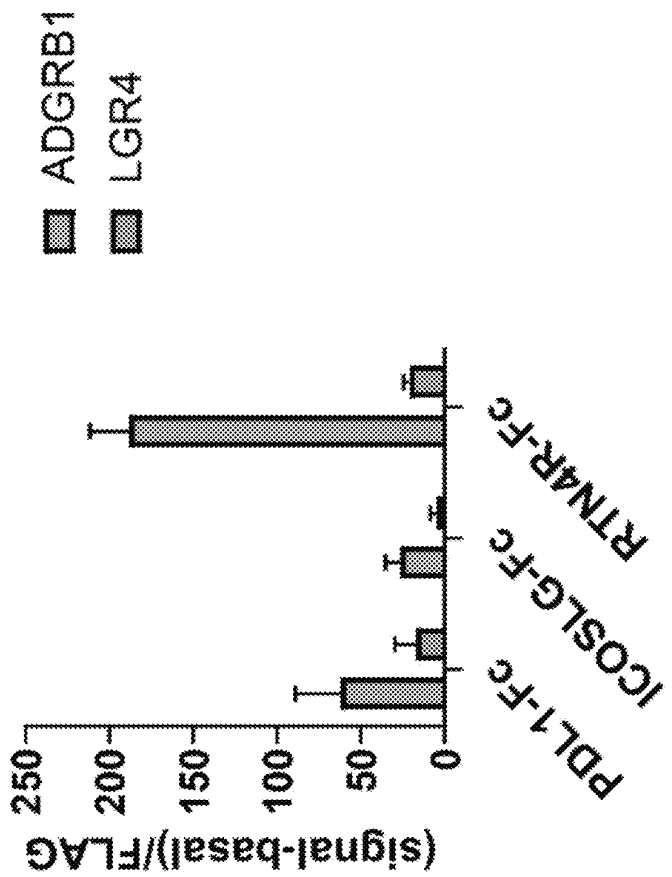

ADGRB1
LGR4
(signal-basal)/FLAG
250
200
150
100
50
0
PDL1-Fc
ICOSLG-Fc
RTN4R-Fc

FIG. 22A

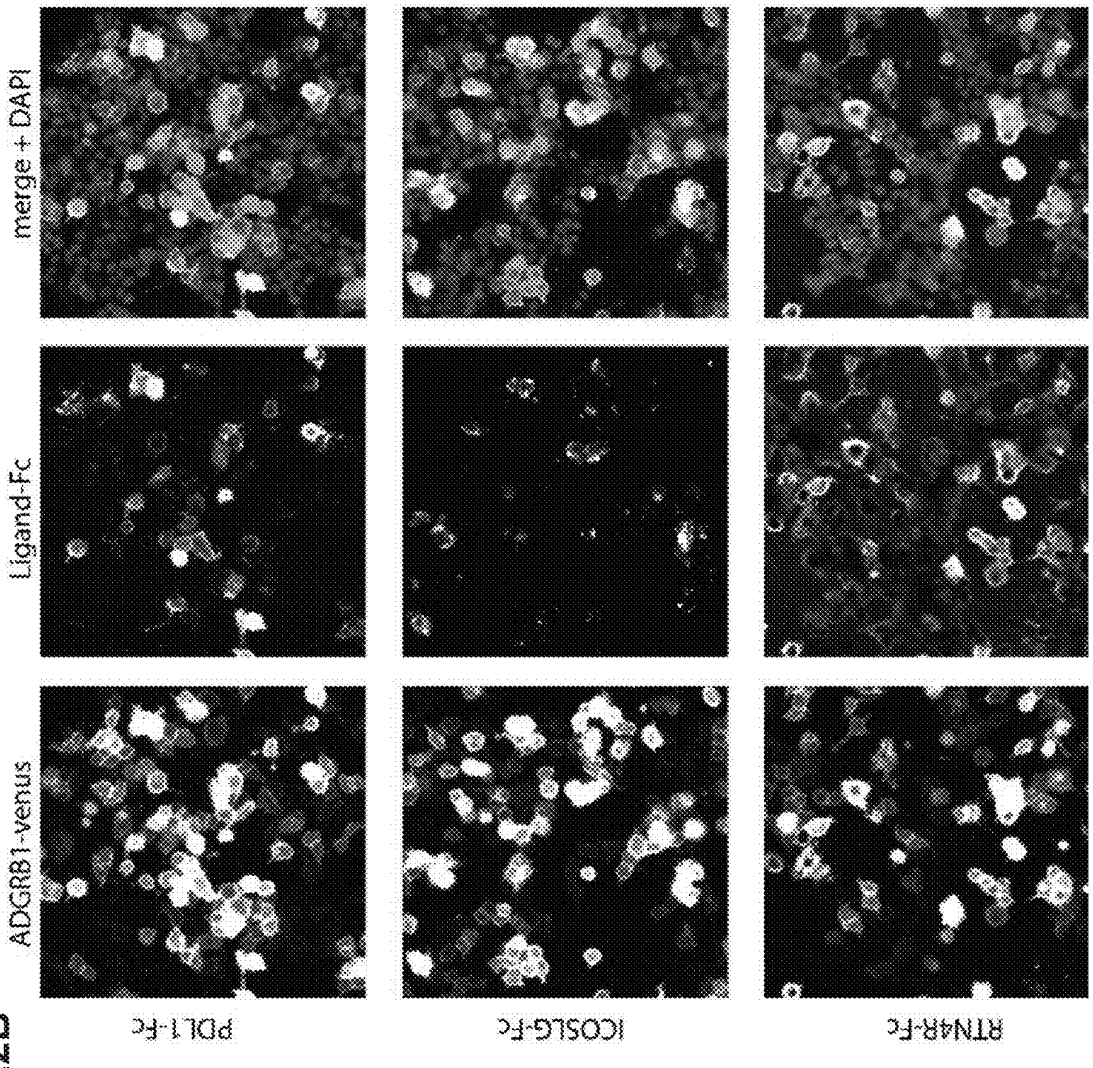


FIG. 22B

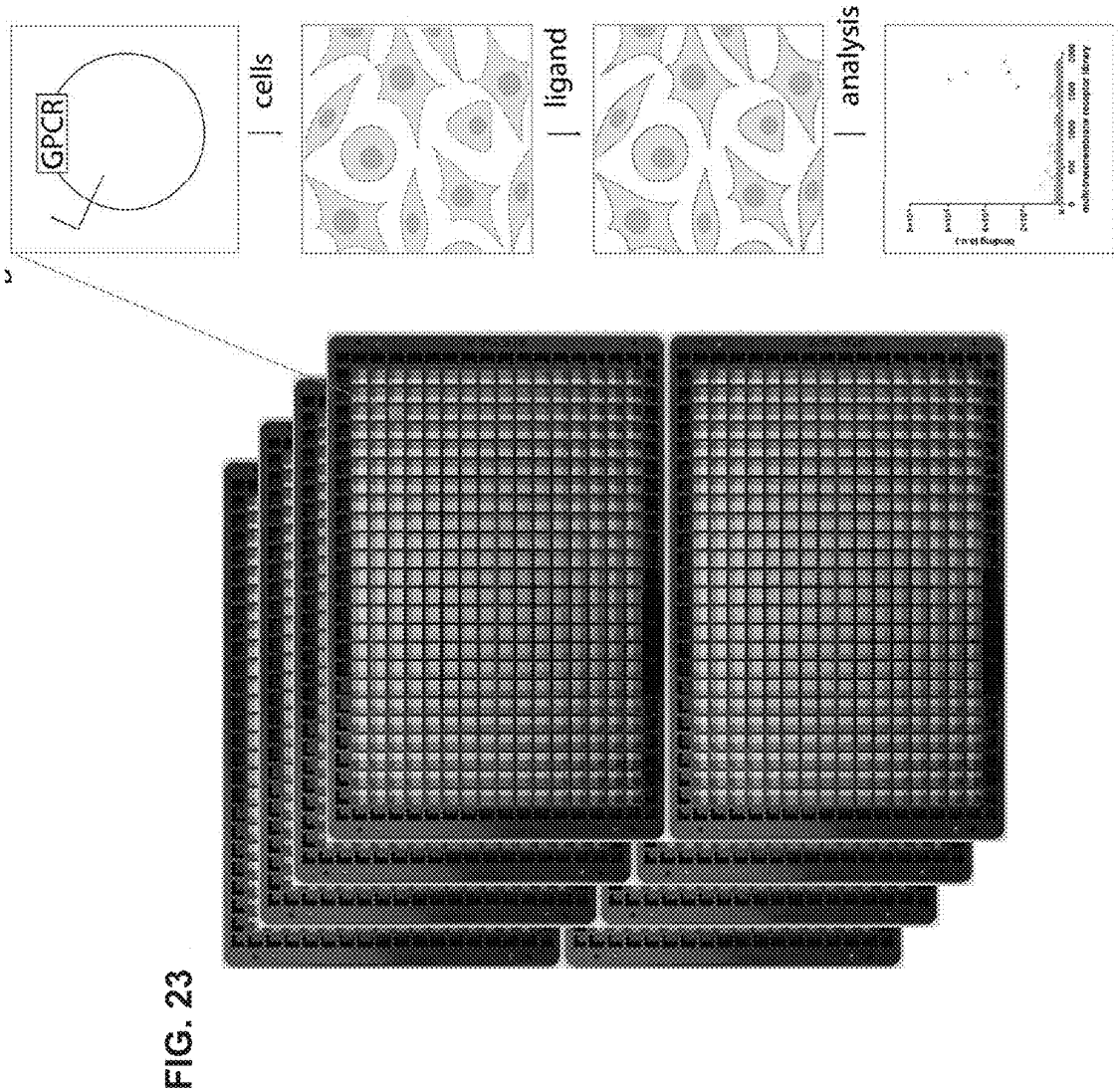


FIG. 23

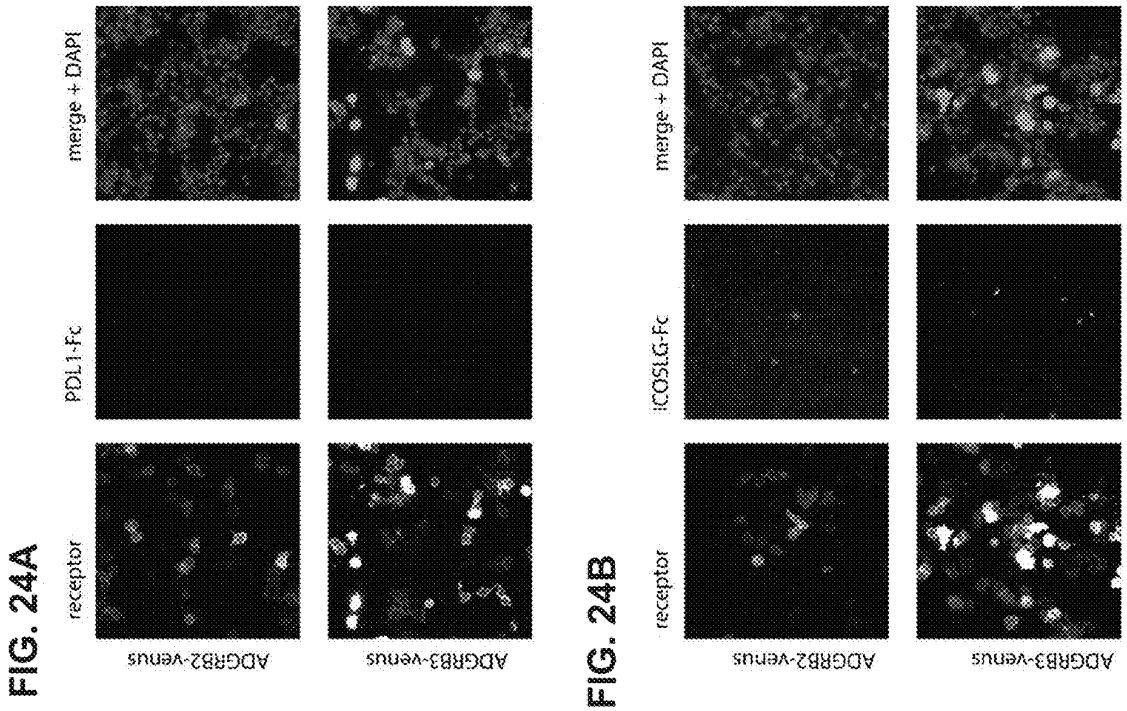


FIG. 24A
receptor
PDL1-Fc
merge + DAPI
ADGRB2-venus
ADGRB3-venus
FIG. 24B
receptor
ICOSLG-Fc
merge + DAPI
ADGRB2-venus
ADGRB3-venus
FIG. 24C
receptor
RTN4R-Fc
merge + DAPI
ADGRB2-venus
ADGRB3-venus

FIG. 25A

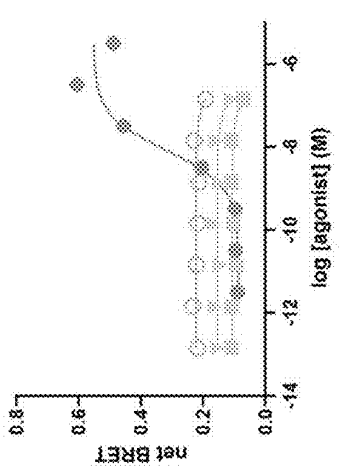


FIG. 25B

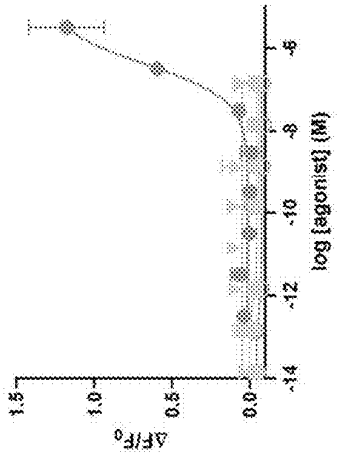


FIG. 25C

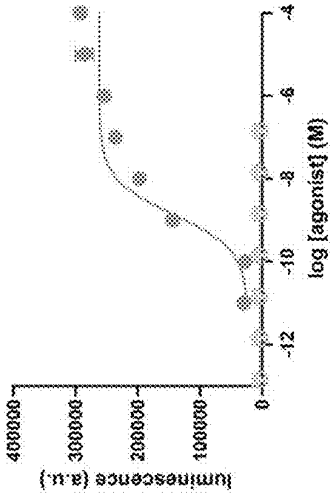
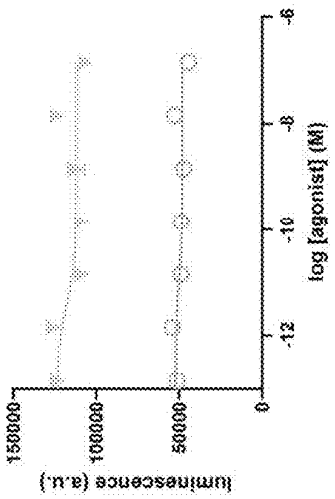


FIG. 25D



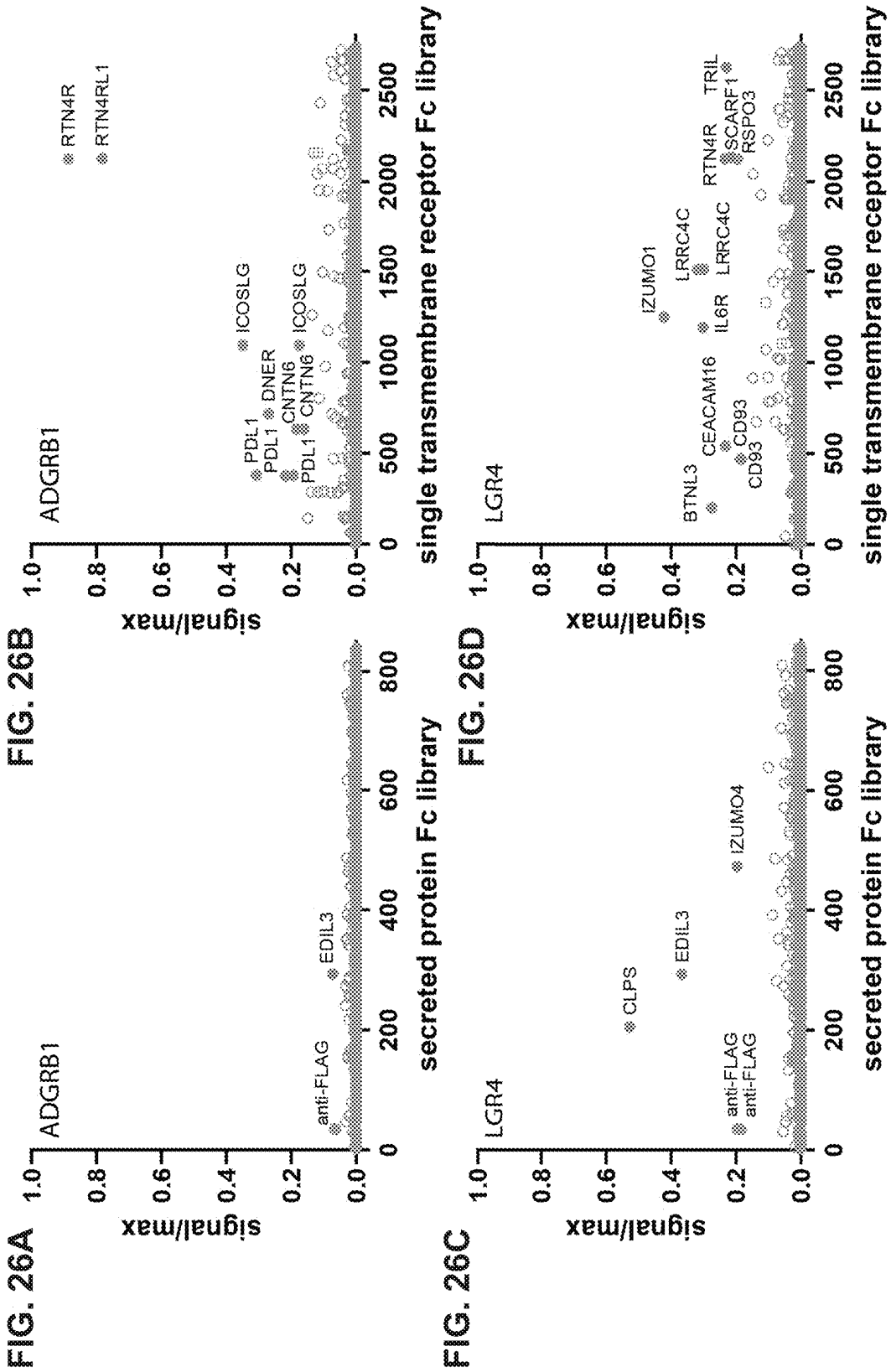


FIG. 26A
ADGRB1
signal/max
anti-FLAG
EDIL3
secreted protein Fc library
FIG. 26B
ADGRB1
signal/max
RTN4R
RTN4RL1
ICOSLG
PDL1
DNER
CNTN6
single transmembrane receptor Fc library
FIG. 26C
LGR4
signal/max
anti-FLAG
CLPS
EDIL3
IZUMO4
secreted protein Fc library
FIG. 26D
LGR4
signal/max
IZUMO1
LRRC4C
BTNL3
CEACAM16
IL6R
CD93
RTN4R
TRIL
SCARF1
RSPO3
single transmembrane receptor Fc library

FIG. 26E

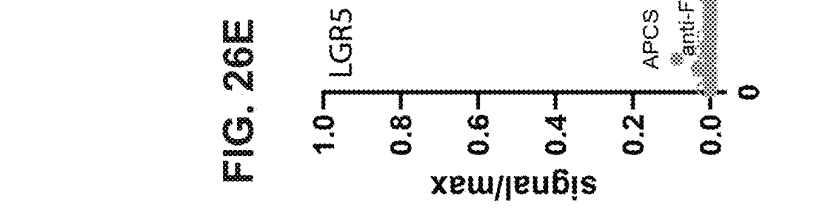
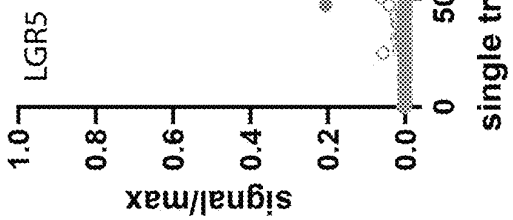


FIG. 26F



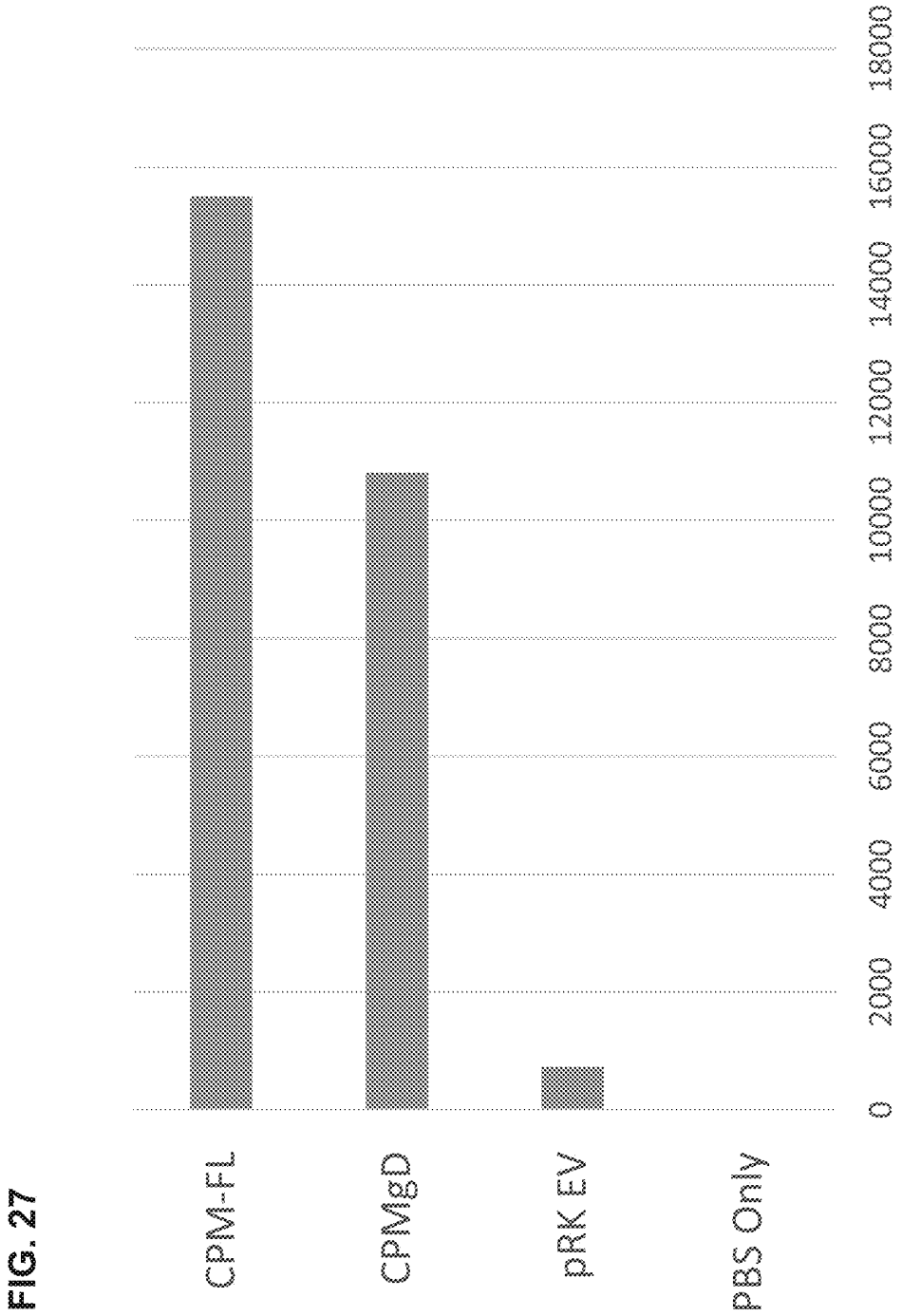

0
2000
4000
6000
8000
10000
12000
14000
16000
18000
CPM-FL
CPMgD
pRK EV
PBS Only

FIG. 27

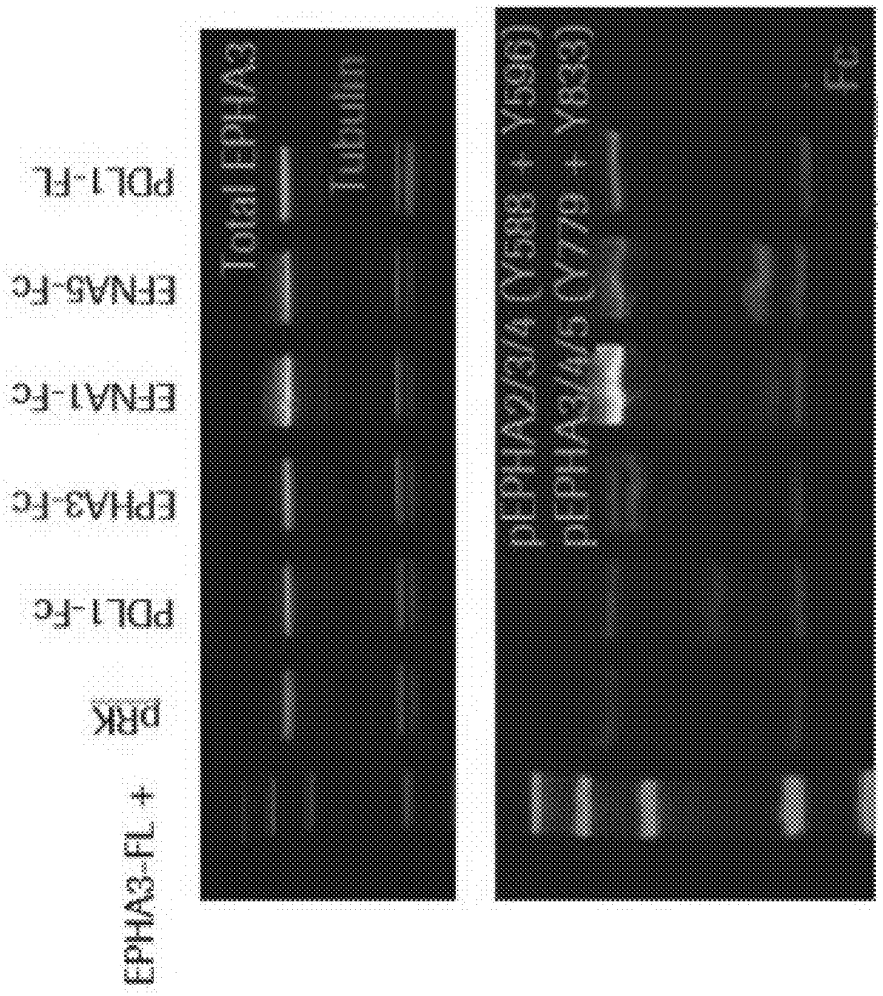

EPHA3-FL +
DRK
PDL1-Fc
EPHA3-Fc
EFNA1-Fc
EFNA5-Fc
PDL1-FL
IP:anti-EPHA3
WB:anti-pY
WB:anti-EPHA3
IP:anti-pY
WB:anti-EPHA3

FIG. 28

BIOLOGICAL VESICLES DISPLAYING CELL SURFACE PROTEINS AND METHODS RELATED TO SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of International Patent Application No. PCT/US2021/061120, filed on Nov. 30, 2021, which claims benefit to U.S. Patent Application No. 63/120,167, filed on Dec. 1, 2020; U.S. Patent Application No. 63/212,021, filed on Jun. 17, 2021; and U.S. Patent Application No. 63/227,039, filed on Jul. 29, 2021, the entire contents of each of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on May 12, 2023 is named 50474-231005_Sequence_Listing_5_10_23.xml and is 2,293 bytes in size.

FIELD OF THE INVENTION

[0003] Provided herein are biological vesicles displaying cell surface proteins, as well as methods of using such vesicles to identify and characterize protein-protein interactions.

BACKGROUND

[0004] Plasma membrane-expressed proteins and their interactors play a prominent role in the initiation of signal transduction to the cytosol of the cell, and thus are key regulators of most biological pathways. Increasing evidence demonstrates that receptors have a complex landscape of interacting partners in the extracellular milieu that directly influence their biological functions. As a result, dysregulation of receptor-ligand crosstalk often underlies pathology and disease progression. However, receptor interaction networks remain understudied, due to of the biochemical challenges associated with maintaining membrane proteins in their native conformation proteins and typically weak interactions among receptors.

[0005] Thus, there is an unmet need for methods and compositions for the identification of interactions between cell surface proteins, as well as novel modulators of such interactions and methods of using the same.

SUMMARY OF THE INVENTION

[0006] In one aspect, the disclosure features a method for identifying a protein-protein interaction, the method comprising (a) providing a collection of target polypeptides that are immobilized on one or more solid surfaces; (b) contacting the collection of step (a) with a biological vesicle (BV) comprising a heterologous membrane-associated protein and a membrane-budding agent under conditions permitting the binding of the heterologous membrane-associated protein and at least one of the target polypeptides, wherein the heterologous membrane-associated protein is expressed at or above a threshold level on the surface of the BV; and (c) detecting an interaction between the heterologous membrane-associated protein and the at least one target polypeptide, thereby identifying a protein-protein interaction.

[0007] In some aspects, one or more of the target polypeptides is immobilized to a distinct location on the one or more solid surfaces.

[0008] In some aspects, detecting an interaction comprises detecting a signal at a location on the solid surface that is above a threshold level.

[0009] In some aspects, the membrane-budding agent is selected from the group consisting of a HIV gag protein, Acyl.Hrs, ARRDC1, and ARF6. In some aspects, the membrane-budding agent is a HIV gag protein.

[0010] In some aspects, the membrane-budding agent further comprises a detectable marker, and detecting an interaction comprises detecting a level of the detectable marker at a location on the solid surface that is above a threshold level. In some aspects, the detectable marker is an enzyme that produces a fluorescent signal in the presence of a substrate. In some aspects, the enzyme is *Renilla* luciferase (Rluc) and the substrate is Rluc substrate.

[0011] In some aspects, the BV comprises a membrane marker, and detecting an interaction comprises detecting a level of the membrane marker at a location on the solid surface that is above a threshold level.

[0012] In some aspects, the membrane marker is a cholesterol marker. In some aspects, the cholesterol marker is AMPLEX™ Red.

[0013] In some aspects, the interaction is a transient interaction.

[0014] In some aspects, the interaction is a low-affinity interaction.

[0015] In some aspects, the heterologous membrane-associated protein is a full-length protein.

[0016] In some aspects, the heterologous membrane-associated protein comprises a protein fragment, a tag, and an anchor.

[0017] In some aspects, the anchor tethers the protein fragment to the surface of a membrane of a BV.

[0018] In some aspects, the anchor is a glycosylphosphatidyl-inositol (GPI) polypeptide.

[0019] In some aspects, the tag can be directly or indirectly visualized. In some aspects, the tag comprises a moiety that can be detected using an antibody or an antibody fragment. In some aspects, the tag is a glycoprotein D (gD) polypeptide.

[0020] In some aspects, the expression level of the heterologous membrane-associated protein is determined using a biolayer interferometry (BLI) assay.

[0021] In some aspects, the tag is a gD polypeptide, expression of the heterologous membrane-associated protein is detected using an anti-gD antibody, and the threshold level is a shift of 1.5 nm, as measured using the BLI assay at 30° C.

[0022] In some aspects, the tag comprises a fluorescent protein.

[0023] In some aspects, the heterologous membrane-associated protein is a transmembrane receptor or a fragment thereof. In some aspects, the receptor is a single-pass transmembrane (STM) receptor.

[0024] In some aspects, the protein fragment is an extracellular domain.

[0025] In some aspects, each member of the collection of target polypeptides is an Fc-tagged extracellular domain, and wherein the solid surface is coated with protein A.

[0026] In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 25% of

the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 50% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 75% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 90% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of all of the proteins of Table 4.

[0027] In another aspect, the disclosure features a BV comprising (a) a heterologous membrane-associated protein comprising a protein fragment, a tag, and an anchor, wherein the heterologous membrane-associated protein is present on the outer face of the BV and (b) a membrane-budding agent.

[0028] In another aspect, the disclosure features a BV comprising (a) a heterologous membrane-associated protein comprising a protein fragment, a tag, and an anchor, wherein the heterologous membrane-associated protein is present on the outer face of the BV and (b) a membrane-budding agent, the BV being produced by a process comprising (i) providing a parent cell that has been modified to express the heterologous membrane-associated protein and the membrane-budding agent and (ii) isolating the BV from the parent cell.

[0029] In some aspects, the membrane-budding agent is selected from the group consisting of a HIV gag protein, Acyl.Hrs, ARRDC1, and ARF6. In some aspects, the membrane-budding agent is a HIV gag protein.

[0030] In some aspects, the anchor tethers the protein fragment to the surface of a lipid membrane of a BV. In some aspects, the anchor is a GPI polypeptide.

[0031] In some aspects, the tag can be directly or indirectly visualized. In some aspects, the tag comprises a moiety that can be detected using an antibody or an antibody fragment. In some aspects, the tag is a gD polypeptide.

[0032] In some aspects, the tag comprises a fluorescent protein.

[0033] In some aspects, the protein fragment is an extracellular domain of a transmembrane receptor. In some aspects, the transmembrane receptor is a STM receptor.

[0034] In some aspects, the BV produces a shift that is at or above a threshold level when contacted with an antibody against the tag, as measured using a BLI assay.

[0035] In some aspects, the tag is a gD polypeptide, the antibody is an anti-gD antibody, and the threshold level is a shift of 1.5 nm, as measured using the BLI assay at 30° C.

[0036] In some aspects, the membrane-budding agent comprises a detectable marker. In some aspects, the detectable marker is an enzyme that produces a fluorescent signal in the presence of a substrate. In some aspects, the enzyme is Rluc and the substrate is Rluc substrate.

[0037] In some aspects, the BV comprises a membrane marker. In some aspects, the membrane marker is a cholesterol marker. In some aspects, the cholesterol marker is AMPLEX™ Red.

[0038] In some aspects, the BV is produced by a mammalian parent cell. In some aspects, the BV is an extracellular vesicle (EV). In some aspects, the BV is an exosome or a microvesicle. In some aspects, the BV is a virus-like particle (VLP).

[0039] In some aspects, the parent cell has been transfected with a plasmid encoding the heterologous membrane-associated protein and a plasmid encoding the membrane-budding agent.

[0040] In another aspect, the disclosure features a method of identifying a modulator of the interaction between a protein of Table 1 and a protein of Table 2, the method comprising (a) providing a candidate modulator; (b) contacting a protein of Table 1 with a protein of Table 2 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 1 to the protein of Table 2, wherein the protein of Table 1 and the protein of Table 2 are reported to interact in Table 3; and (c) measuring the binding of the protein of Table 1 to the protein of Table 2, wherein an increase or decrease in binding in the presence of the candidate modulator relative to binding in the absence of the candidate modulator identifies the candidate modulator as a modulator of the interaction between the protein of Table 1 and the protein of Table 2.

[0041] In another aspect, the disclosure features a method of identifying a modulator of a downstream activity of a protein of Table 1, the method comprising (a) providing a candidate modulator; (b) contacting the protein of Table 1 with a protein of Table 2 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 1 to the protein of Table 2, wherein the protein of Table 1 and the protein of Table 2 are reported to interact in Table 3; and (c) measuring a downstream activity of the protein of Table 1, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of the protein of Table 1.

[0042] In another aspect, the disclosure features a method of identifying a modulator of a downstream activity of a protein of Table 2, the method comprising (a) providing a candidate modulator; (b) contacting the protein of Table 2 with a protein of Table 1 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 2 to the protein of Table 1, wherein the protein of Table 1 and the protein of Table 2 are reported to interact in Table 3; and (c) measuring a downstream activity of the protein of Table 2, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of the protein of Table 2.

[0043] In some aspects, the increase or decrease in binding is at least 70%, as measured by a surface plasmon resonance (SPR) assay, a BLI assay, or an enzyme-linked immunosorbent assay (ELISA).

[0044] In some aspects, the modulator is an inhibitor of the downstream activity of the protein of Table 1 or Table 2. In some aspects, the modulator is an activator of the downstream activity of the protein of Table 1 or Table 2.

[0045] In some aspects, the change in the downstream activity is a decrease in the amount, strength, or duration of the downstream activity. In some aspects, the change in the downstream activity is an increase in the amount, strength, or duration of the downstream activity.

[0046] In some aspects, the modulator is a small molecule, an antibody or antigen-binding fragment thereof, a peptide, a mimic, an antisense oligonucleotide, or a small interfering RNA (siRNA).

[0047] In some aspects, the antigen-binding fragment is a bis-Fab, an Fv, a Fab, a Fab'-SH, a $F(ab')_2$, a diabody, a linear antibody, an scFv, an ScFab, a VH domain, or a VHH domain.

[0048] In some aspects, the antibody or antigen-binding fragment thereof binds the protein of Table 1. In some aspects, the antibody or antigen-binding fragment thereof binds the protein of Table 2.

[0049] In some aspects, the protein of Table 1 is LRRC15. In some aspects, the protein of Table 2 is TEM1. In some aspects, the downstream activity is tumor growth. In some aspects, tumor growth is decreased in the presence of the modulator. In some aspects, tumor growth is decreased by at least 20%, as measured in a tumor growth assay.

[0050] In some aspects, the modulator is an antibody or antigen-binding fragment thereof targeting LRRC15.

[0051] In some aspects, the modulator is an antibody or antigen-binding fragment thereof targeting TEM1.

[0052] In another aspect, the disclosure features a method of identifying a modulator of the interaction between LRRC15 and TEM1, the method comprising (a) providing a candidate modulator; (b) contacting LRRC15 with TEM1 in the presence or absence of the candidate modulator under conditions permitting the binding of LRRC15 to TEM1; and (c) measuring the binding of LRRC15 to TEM1, wherein an increase or decrease in binding in the presence of the candidate modulator relative to binding in the absence of the candidate modulator identifies the candidate modulator as a modulator of the interaction between LRRC15 and TEM1.

[0053] In another aspect, the disclosure features a method of identifying a modulator of a downstream activity of LRRC15, the method comprising (a) providing a candidate modulator; (b) contacting LRRC15 with TEM1 in the presence or absence of the candidate modulator under conditions permitting the binding of LRRC15 to TEM1; and (c) measuring a downstream activity of LRRC15, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of LRRC15.

[0054] In another aspect, the disclosure features a method of identifying a modulator of a downstream activity of TEM1, the method comprising (a) providing a candidate modulator; (b) contacting TEM1 with LRRC15 in the presence or absence of the candidate modulator under conditions permitting the binding of TEM1 to LRRC15; and (c) measuring a downstream activity of TEM1, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of TEM1.

[0055] In some aspects, the increase or decrease in binding is at least 70%, as measured by an SPR assay, a BLI assay, or ELISA.

[0056] In some aspects, the downstream activity is tumor growth.

[0057] In some aspects, tumor growth is decreased in the presence of the modulator. In some aspects, tumor growth is decreased by at least 20%, as measured in a tumor growth assay.

[0058] In another aspect, the disclosure features a method for identifying a biological vesicle (BV) having an altered binding profile, the method comprising (a) providing a collection of target polypeptides that are immobilized on one or more solid surfaces; (b) contacting the collection of step (a) with a BV of interest; (c) detecting an interaction between the BV of interest and the at least one target polypeptide, thereby identifying an interaction profile; and (d) comparing the interaction profile of the BV of interest to the interaction profile of a control BV, wherein a difference between the interaction profile of the BV of interest and the interaction profile of the control BV identifies the BV of interest as one having an altered binding profile.

[0059] In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 25% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 50% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 75% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 90% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of all of the proteins of Table 4.

[0060] In some aspects, the BV of interest is an engineered BV.

[0061] In some aspects, the BV of interest is derived from a sample from a subject. In some aspects, the BV of interest and the control BV are derived from different tissues or different cell types. In some aspects, the BV of interest is derived from a diseased tissue and the control BV is derived from healthy tissue.

[0062] In another aspect, the disclosure features a protein complex comprising (a) a BV comprising a heterologous membrane-associated protein and a membrane-budding agent and (b) a target polypeptide, wherein the heterologous membrane-associated protein and the target polypeptide are bound to one another.

[0063] In another aspect, the disclosure features a method of identifying a modulator of the interaction between a protein of Table 5 and a protein of Table 6, the method comprising (a) providing a candidate modulator; (b) contacting a protein of Table 5 with a protein of Table 6 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 5 to the protein of Table 6, wherein the protein of Table 5 and the protein of Table 6 are reported to interact in Table 7; and (c) measuring the binding of the protein of Table 5 to the protein of Table 6, wherein an increase or decrease in binding in the presence of the candidate modulator relative to binding in the absence of the candidate modulator identifies the candidate modulator as a modulator of the interaction between the protein of Table 5 and the protein of Table 6.

[0064] In another aspect, the disclosure features a method of identifying a modulator of a downstream activity of a protein of Table 5, the method comprising (a) providing a candidate modulator; (b) contacting the protein of Table 5 with a protein of Table 6 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 5 to the protein of Table 6, wherein the protein of Table 5 and the protein of Table 6 are reported to interact in Table 7; and (c) measuring a downstream activity of the protein of Table 5, wherein a change

in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of the protein of Table 5.

[0065] In another aspect, the disclosure features a method of identifying a modulator of a downstream activity of a protein of Table 6, the method comprising (a) providing a candidate modulator; (b) contacting the protein of Table 6 with a protein of Table 5 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 6 to the protein of Table 5, wherein the protein of Table 5 and the protein of Table 6 are reported to interact in Table 7; and (c) measuring a downstream activity of the protein of Table 6, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of the protein of Table 6.

[0066] In some aspects, the increase or decrease in binding is at least 70%, as measured by a surface plasmon resonance (SPR) assay, a BLI assay, or an enzyme-linked immunosorbent assay (ELISA).

[0067] In some aspects, the modulator is an inhibitor of the downstream activity of the protein of Table 5 or Table 6.

[0068] In some aspects, the modulator is an activator of the downstream activity of the protein of Table 5 or Table 6.

[0069] In some aspects, the change in the downstream activity is a decrease in the amount, strength, or duration of the downstream activity. In some aspects, the change in the downstream activity is an increase in the amount, strength, or duration of the downstream activity.

[0070] In some aspects, the modulator is a small molecule, an antibody or antigen-binding fragment thereof, a peptide, a mimic, an antisense oligonucleotide, or a small interfering RNA (siRNA).

[0071] In some aspects, the antigen-binding fragment is a bis-Fab, an Fv, a Fab, a Fab'-SH, a $F(ab')_2$, a diabody, a linear antibody, an scFv, an ScFab, a VH domain, or a VHH domain.

[0072] In some aspects, the antibody or antigen-binding fragment thereof binds the protein of Table 5.

[0073] In some aspects, the antibody or antigen-binding fragment thereof binds the protein of Table 6.

[0074] In some aspects, the protein of Table 5 is ADGRB1.

[0075] In some aspects, the protein of Table 6 is PD-L1.

[0076] In some aspects, the downstream activity is tumor growth. In some aspects, tumor growth is decreased in the presence of the modulator. In some aspects, tumor growth is decreased by at least 20%, as measured in a tumor growth assay.

[0077] In some aspects, the modulator is an antibody or antigen-binding fragment thereof targeting PD-L1.

[0078] In some aspects, the protein of Table 6 is ICOSLG.

[0079] In some aspects, the downstream activity is T cell activation. In some aspects, T cell activation is increased in the presence of the modulator. In some aspects, T cell activation is increased by at least 20%.

[0080] In some aspects, the modulator is an antibody or antigen-binding fragment thereof targeting ICOSLG.

[0081] In some aspects, the modulator is an antibody or antigen-binding fragment thereof targeting ADGRB1.

[0082] In another aspect, the disclosure features a method of identifying a modulator of the interaction between PD-L1 and ADGRB1, the method comprising (a) providing a candidate modulator; (b) contacting PD-L1 with ADGRB1 in the presence or absence of the candidate modulator under conditions permitting the binding of PD-L1 to ADGRB1; and (c) measuring the binding of PD-L1 to ADGRB1, wherein an increase or decrease in binding in the presence of the candidate modulator relative to binding in the absence of the candidate modulator identifies the candidate modulator as a modulator of the interaction between PD-L1 and ADGRB1.

[0083] In another aspect, the disclosure features a method of identifying a modulator of a downstream activity of PD-1, the method comprising (a) providing a candidate modulator; (b) contacting PD-L1 with ADGRB1 in the presence or absence of the candidate modulator under conditions permitting the binding of PD-L1 to ADGRB1; and (c) measuring a downstream activity of PD-L1, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of PD-1.

[0084] In another aspect, the disclosure features a method of identifying a modulator of a downstream activity of ADGRB1, the method comprising (a) providing a candidate modulator; (b) contacting ADGRB1 with PD-L1 in the presence or absence of the candidate modulator under conditions permitting the binding of ADGRB1 to PD-L1; and (c) measuring a downstream activity of ADGRB1, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of ADGRB1.

[0085] In some aspects, the increase or decrease in binding is at least 70%, as measured by an SPR assay, a BLI assay, or ELISA.

[0086] In some aspects, the downstream activity is tumor growth. In some aspects, tumor growth is decreased in the presence of the modulator. In some aspects, tumor growth is decreased by at least 20%, as measured in a tumor growth assay.

[0087] In another aspect, the disclosure features a method of identifying a modulator of the interaction between ICOSLG and ADGRB1, the method comprising (a) providing a candidate modulator; (b) contacting ICOSLG with ADGRB1 in the presence or absence of the candidate modulator under conditions permitting the binding of ICOSLG to ADGRB1; and (c) measuring the binding of ICOSLG to ADGRB1, wherein an increase or decrease in binding in the presence of the candidate modulator relative to binding in the absence of the candidate modulator identifies the candidate modulator as a modulator of the interaction between ICOSLG and ADGRB1.

[0088] In another aspect, the disclosure features a method of identifying a modulator of a downstream activity of ICOSLG, the method comprising (a) providing a candidate modulator; (b) contacting ICOSLG with ADGRB1 in the presence or absence of the candidate modulator under conditions permitting the binding of ICOSLG to ADGRB1; and (c) measuring a downstream activity of ICOSLG, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in

the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of ICOSLG.

[0089] In another aspect, the disclosure features a method of identifying a modulator of a downstream activity of ADGRB1, the method comprising (a) providing a candidate modulator; (b) contacting ADGRB1 with ICOSLG in the presence or absence of the candidate modulator under conditions permitting the binding of ADGRB1 to ICOSLG; and (c) measuring a downstream activity of ADGRB1, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of ADGRB1.

[0090] In some aspects, the increase or decrease in binding is at least 70%, as measured by an SPR assay, a BLI assay, or ELISA.

[0091] In some aspects, the downstream activity is T cell activation. In some aspects, T cell activation is decreased in the presence of the modulator. In some aspects, T cell activation is increased by at least 20%.

[0092] In another aspect, the disclosure features a method for characterizing an interaction profile of a cell line, the method comprising (a) modifying the cell line to comprise a membrane-budding agent; and (b) characterizing an interaction profile of a biological vesicle (BV) produced by the cell line.

[0093] In another aspect, the disclosure features a method for characterizing an interaction profile of a cell line that has been modified to comprise a membrane-budding agent, the method comprising characterizing an interaction profile of a BV produced by the cell line.

[0094] In another aspect, the disclosure features a method for identifying a change in the interaction profile of a cell line, the method comprising (a) modifying the cell line to comprise a membrane-budding agent; (b) characterizing an interaction profile of a BV produced by the cell line at a first time point; (c) characterizing an interaction profile of a BV produced by the cell line at a second time point; and (d) comparing the interaction profile of the BV produced at the first time point to that of the BV produced at the second time point, wherein a difference between the interaction profile of the BV produced at the first time point and that of the BV produced at the second time point identifies a change in the interaction profile of the cell line.

[0095] In another aspect, the disclosure features a method for identifying a change in the interaction profile of a cell line that has been modified to comprise a membrane-budding agent, the method comprising (a) characterizing an interaction profile of a BV produced by the cell line at a first time point; (b) characterizing an interaction profile of a BV produced by the cell line at a second time point; and (c) comparing the interaction profile of the BV produced at the first time point to that of the BV produced at the second time point, wherein a difference between the interaction profile of the BV produced at the first time point and that of the BV produced at the second time point identifies a change in the interaction profile of the cell line.

[0096] In some aspects, the cell line is a mammalian cell line. In some aspects, the mammalian cell line is an immune cell line, a neuronal cell line, or a fibroblast cell line. In some aspects, the immune cell line comprises one or more of T-cells, B-cells, or monocytes.

[0097] In some aspects, the method comprises exposing the cell line to a stimulus following the first time point and before the second time point.

[0098] In some aspects, the stimulus is a condition or agent that induces signaling. In some aspects, the stimulus is a condition or agent that induces a disease-related state. In some aspects, the cell line is an immune cell line and the disease-related state is immune exhaustion.

[0099] In some aspects, the stimulus is a condition or agent that induces differentiation.

[0100] In some aspects, the method further comprises characterizing an interaction profile of a BV produced by the cell line at one or more additional time points.

[0101] In another aspect, the disclosure features a method for identifying a difference in the interaction profiles of two cell lines, the method comprising (a) modifying each of the cell lines to comprise a membrane-budding agent; (b) characterizing an interaction profile of a BV produced by the first cell line; (c) characterizing an interaction profile of a BV produced by the second cell line; and (d) comparing the interaction profile of the BV produced at the first cell line to that of the BV produced by the second cell line, wherein a difference between the interaction profile of the BV produced by the first cell line and that of the BV produced by the second cell line identifies a difference in the surface protein profiles of two cell lines.

[0102] In another aspect, the disclosure features a method for identifying a difference in the interaction profiles of two cell lines that have been modified to comprise a membrane-budding agent, the method comprising (a) characterizing an interaction profile of a BV produced by the first cell line; (b) characterizing an interaction profile of a BV produced by the second cell line; and (c) comparing the interaction profile of the BV produced at the first cell line to that of the BV produced by the second cell line, wherein a difference between the interaction profile of the BV produced by the first cell line and that of the BV produced by the second cell line identifies a difference in the surface protein profiles of two cell lines.

[0103] In some aspects, expression of the membrane-budding agent is inducible.

[0104] In some aspects, characterizing the interaction profile of the BV comprises determining a level of one or more membrane-associated proteins of interest on the BV.

[0105] In some aspects, characterizing the interaction profile of the BV comprises determining a level of one or more receptors of interest on the BV.

[0106] In some aspects, characterizing the interaction profile of the BV is performed using a method comprising (a) providing a collection of target polypeptides that are immobilized on one or more solid surfaces; (b) contacting the collection of target polypeptides in step (a) with the BV; and (c) detecting an interaction between the BV and the at least one target polypeptide of the collection of target polypeptides, thereby identifying an interaction profile.

[0107] In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 25% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 50% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 75% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 90% of the proteins of Table

4. In some aspects, the collection of target polypeptides comprises the extracellular domains of all of the proteins of Table 4.

[0108] In some aspects, the method further comprises characterizing a cytoplasmic protein profile of the BV.

[0109] In some aspects, the membrane-budding agent is selected from the group consisting of a HIV gag protein, Acyl.Hrs, ARRDC1, and ARF6.

[0110] In another aspect, the disclosure features a BV comprising a heterologous membrane-budding agent, wherein the BV is produced by a process comprising (i) providing a parent cell line that has been modified to express the membrane-budding agent under inducible control; (ii) inducing expression of the membrane-budding agent, and (iii) isolating the BV from the parent cell line.

[0111] In some aspects, the membrane-budding agent is selected from the group consisting of a HIV gag protein, Acyl.Hrs, ARRDC1, and ARF6.

[0112] In some aspects, the parent cell line is a mammalian cell line.

[0113] In some aspects, the BV is an extracellular vesicle (EV).

[0114] In another aspect, the disclosure features a method for assessing an enzymatic activity of a membrane-associated protein, the method comprising conducting an assay for enzymatic activity on a BV comprising the protein.

[0115] In some aspects, the membrane-associated protein is a peptidase and the assay for enzymatic activity is an assay for peptidase activity.

[0116] In some aspects, the membrane-associated protein is a protease and the assay for enzymatic activity is an assay for protease activity.

[0117] In some aspects, the membrane-associated protein is a kinase and the assay for enzymatic activity is an assay for kinase activity.

[0118] In some aspects, the membrane-associated protein is a phosphatase and the assay for enzymatic activity is an assay for phosphatase activity.

[0119] In some aspects, the membrane-associated protein is endogenous to a parent cell from which the BV is derived.

[0120] In some aspects, the membrane-associated protein is heterologous to a parent cell from which the BV is derived. In some aspects, the heterologous membrane-associated protein is a full-length protein. In some aspects, the heterologous membrane-associated protein comprises a protein fragment, a tag, and an anchor. In some aspects, the anchor tethers the protein fragment to the surface of a membrane of the BV. In some aspects, the anchor is a glycosylphosphatidyl-inositol (GPI) polypeptide.

[0121] In another aspect, the disclosure features a method of purifying a BV from a culture medium or a sample from a subject, the method comprising contacting a BV with a solid surface comprising one or more of the proteins of Table 8 or Table 9, wherein the one or more proteins of Table 8 or Table 9 have been modified to comprise an Fc region.

[0122] In some aspects, the sample from the subject is a urine sample, a blood sample, or a digested tissue sample

[0123] In some aspects, the solid surface is a column comprising Protein A-functionalized beads and the method comprises flowing the conditioned media comprising the one or more of the proteins of Table 8 or Table 9 over the column.

[0124] In some aspects, the method further comprises flowing the culture medium comprising the BV over the column.

[0125] In some aspects, the method further comprises eluting the BV.

BRIEF DESCRIPTION OF THE DRAWINGS

[0126] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0127] FIG. **1**A is a schematic diagram showing the isolation of receptor-expressing extracellular vesicles (EVs) from cell culture. EXPI293F™ cells were transiently transfected with a plasmid encoding a receptor of interest and a plasmid encoding an HIV gag protein fused to *Renilla* luciferase (Rluc). Cells and debris were separated from the EV-containing supernatant by centrifugation and filtration. A 50% sucrose cushion was used to remove small protein aggregates, and small vesicles were isolated.

[0128] FIG. **1**B is a set of negative stain electron micrographs showing EVs prepared with (right panel) and without (left panel) the sucrose cushion step. EV preparations were diluted to the same protein concentration prior to electron microscopy. Arrows point to representative EVs in the sample.

[0129] FIG. **1**C is a pair of graphs showing the size distribution (in nm) of EVs carrying a full-length (FL) PVR protein (left panel) or a protein comprising the PVR ectodomain, a glycoprotein D (gD) tag, and a glycosylphosphatidylinositol (GPI) linker (gD-GPI) (right panel), as measured using nanoparticle tracking analysis. Five replicates are shown in each graph. Black line represents the mean; gray line represents the standard error of the mean. EVs are consistently about 120 nm in size.

[0130] FIG. **2**A is a diagram showing transmembrane proteins embedded in the plasma membrane of the cell and in the EV membrane.

[0131] FIG. **2**B is a diagram showing two experimental setups for EV-expressed receptors. Left: HIV gag proteins and full-length transmembrane receptors embedded in a membrane of the cell and in EV membranes. Right: HIV gag proteins and lipid-anchored ectodomains comprising gD-GPI tags embedded in a membrane of the cell and in EV membranes.

[0132] FIG. **2**C is a graph showing the particle count of EVs in the 20-500 nm size range produced by parent cells that have been transformed with an HIV gag protein (With Gag) and control untransformed cells (No Gag), as measured using nanoparticle tracking analysis.

[0133] FIG. **2**D is a graph showing the luminescence signal of Rluc in a 3-fold dilution series of an EV preparation produced from parent cells that were transformed with a plasmid encoding an HIV gag protein fused to Rluc.

[0134] FIG. **2**E is a schematic diagram showing EVs expressing full-length PVR bound to the surface of a mammalian cell expressing a PVR ligand and a set of micrographs showing EVs bound to the surface of cells expressing the indicated full-length PVR ligands. EVs comprised gag-NeonGreen, and green represents direct fluorescence from the EVs. DNA of the mammalian cells is shown in blue.

[0135] FIG. **2**F is a schematic diagram showing EVs expressing the PVR ectodomain with a gD-GPI tag bound to

the surface of a mammalian cell expressing a PVR ligand and a set of micrographs showing EVs bound to the surface of cells expressing the indicated full-length PVR ligands. EVs comprised gag-NeonGreen, and green represents direct fluorescence from the EVs. DNA of the mammalian cells is shown in blue. Scale bar is 20 μm.

[0136] FIG. **2**G is a schematic diagram and a graph showing the design and results of a biolayer interferometry (BLI) experiment. CD226-Fc or a control human IgG were attached to a sensor. The sensor was dipped into a solution comprising EVs expressing full-length (FL) PVR or gD-GPI PVR ectodomains or monomeric PVR protein (PVR monomer), and the BLI signal (in nm) was measured. Right panel is a zoom of the signal above 0 nm.

[0137] FIG. **3**A is a schematic diagram showing the workflow of the RDIMIS (Receptor-Display In Membranes Interaction Screen) protocol. EVs are isolated from the conditioned media of cells expressing the receptor of interest alongside gag-luc. A library of single-pass transmembrane (STM) proteins, expressed as Fc-tagged ectodomains (ECD-Fc), are immobilized on plates. Receptor-EVs are screened against the collection of plate-bound STM proteins using a semi-automated workflow. EV binding to interacting ectodomains in the library is detected using luminescence.

[0138] FIG. **3**B is a scatter plot showing the results of two independent RDIMIS screens (Repeat 1 and Repeat 2) testing for interaction between PVR gD-GPI EVs and the STM protein library.

[0139] FIG. **3**C is a scatter plot showing the results of an RDIMIS screen testing for interaction between PVR gD-GPI EVs and the STM protein library (Repeat 2 of FIG. **3**B) and an RDIMIS screen testing for interaction between full-length (FL) PVR EVs and the STM protein library.

[0140] FIG. **4**A is a set of photomicrographs showing Western blots of whole cell lysates (Cells) or EVs expressing the full-length untagged receptors PD1, PD-L1, EPHA3, CD248, LRRC15, PVR, or PVRL1 and stained with an antibody specific for the receptor. Anti-tubulin (α-Tub) and anti-actin (α-Actin) staining are provided as controls.

[0141] FIG. **4**B is a set of photomicrographs showing Western blots of whole cell lysates or EVs expressing the indicated gD-GPI tagged receptor ectodomains and stained with an antibody specific for the gD tag (α-gD). Anti-tubulin (α-Tub) and anti-actin (α-Actin) staining are provided as controls.

[0142] FIG. **4**C is a set of negative stain electromicrographs showing selective anti-gD immunogold labeling of gD-GPI expressing vesicles.

[0143] FIG. **4**D is a graph showing the design and results of a biolayer interferometry experiment. An anti-gD antibody was attached to a sensor. The sensor was incubated with EVs expressing the indicated gD-GPI ectodomains, and the BLI signal (in nm) was measured.

[0144] FIG. **5**A is a scatter plot showing the results of an RDIMIS screen testing for interaction between PVR gD-GPI EVs and the STM protein library and an RDIMIS screen testing for interaction between PD-L1 gD-GPI EVs and the STM protein library. Screens are plotted against one another to differentiate receptor-specific hits (near either axis) from the generic vesicle binders common between the screens.

[0145] Hits whose signal is above the 98% quantile for each individual screen and for which there is at least a 4× enrichment for a specific screen are labeled. Other hits are identified as generic vesicle binders common between the screens.

[0146] FIG. **5**B is a scatter plot showing the results of an RDIMIS screen testing for interaction between CD80 gD-GPI EVs and the STM protein library and an RDIMIS screen testing for interaction between CD276 gD-GPI EVs and the STM protein library. Receptor-specific hits are located near the axes. Hits whose signal is above the 98% quantile for each individual screen and for which there is at least a 4× enrichment for a specific screen are labeled. Other hits are identified as generic vesicle binders common between the screens.

[0147] FIG. **5**C is a set of diagrams showing the overlap between the binding partners identified for PVR, PD-L1, CD80, and CD276 in the present study and interactions listed in the STRING, Bioplex and Biogrid databases. For PD-L1/CD274, no interactions with members of the STM library were present in the Bioplex database. No experimentally verified interactions were listed in STRING for CD276/B7-H3.

[0148] FIG. **6**A is a scatter plot showing the results of an AVEXIS screen testing for interaction between LRRC15 ectodomain pentamers and the STM protein library. LRRC15 pentamer binding was not observed above background in the well containing the CD248 ectodomain (highlighted). Gray dots indicate positive control wells on each plate, in which stock pentamer was added but not washed away.

[0149] FIG. **6**B is a set of diagrams showing a comparison between the hits identified herein using LRRC15 full-length (FL) or gD-GPI ectodomain in EVs and those represented in the Bioplex and Biogrid databases. No interaction with experimental evidence between LRRC15 and a STM protein in the library was represented in the STRING database.

[0150] FIG. **7**A is a scatter plot showing the results of an RDIMIS screen testing for interaction between LRRC15 gD-GPI EVs and the STM protein library. Results are compared with PVR screen results shown in FIG. **3**C. Hits whose signal is above the 98% quantile for each individual screen and for which there is at least a 4× enrichment for a specific screen are labeled. Other hits are identified as generic vesicle binders common between the screens.

[0151] FIG. **7**B is a scatter plot showing the results of an RDIMIS screen testing for interaction between LRRC15 full-length EVs and the STM protein library. Results are compared with PVR screen results shown in FIG. **3**C. Hits whose signal is above the 98% quantile for each individual screen and for which there is at least a 4× enrichment for a specific screen are labeled. Other hits are identified as generic vesicle binders common between the screens.

[0152] FIG. **8**A is a scatter plot showing bulk RNA-seq expression levels (transcripts per million (TPM)) of LRRC15 (x-axis) and CD248 (y-axis) for head and neck squamous cell carcinoma. Each point represents a single patient sample. Spearman's rank correlation coefficient and significance values are given on the top right.

[0153] FIG. **8**B is a scatter plot showing bulk RNA-seq expression levels (transcripts per million (TPM)) of LRRC15 (x-axis) and CD248 (y-axis) for breast invasive carcinoma. Each point represents a single patient sample. Spearman's rank correlation coefficient and significance values are given on the top right.

[0154] FIG. **8**C is a pair of Uniform Manifold Approximation and Projection (UMAP) dimensionality reduction plots showing non-tumor cells from single-cell RNA-seq data of head and neck cancer patients. Cells are shaded by expression level of the indicated marker genes (left; LRRC15; right: CD248).

[0155] FIG. **9**A is a scatter plot showing bulk RNA-seq expression levels (transcripts per million (TPM)) of LRRC15 (x-axis) and CD248 (y-axis) for pancreatic ductal adenocarcinoma (The Cancer Genome Atlas (TCGA) data).

[0156] FIG. **9**B is a scatter plot showing bulk RNA-seq expression levels (transcripts per million (TPM)) of LRRC15 (x-axis) and CD248 (y-axis) for urothelial bladder carcinoma (The Cancer Genome Atlas (TCGA) data).

[0157] FIG. **9**C is a pair of UMAP dimensionality reduction plots showing non-tumor cells from single-cell RNA-seq data of head and neck cancer patients. Cells are shaded by expression level of the indicated marker genes (left; DCN cancer-associated fibroblasts; right: RGS5 cancer-associated pericyte markers).

[0158] FIG. **10**A is a scatter plot showing the results of an AVEXIS screen testing for interaction between CD248 ectodomain pentamers and the STM protein library. CD248 pentamer binding was not observed above background in the well containing the LRRC15 ectodomain (highlighted). Gray dots indicate positive control wells on each plate, in which stock pentamer was added but not washed away.

[0159] FIG. **10**B is a graph showing the results of a surface plasmon resonance (SPR) assay in which LRRC15-Fc was captured by Protein A on the SPR chip and the indicated analytes were added. LRRC15 was loaded at either 5 μg/mL (red and green lines) or 50 μg/mL concentration. Analytes were loaded at 400 nM concentration.

[0160] FIG. **11**A is a schematic diagram and a graph showing the design and results of a BLI experiment. CD248 was expressed as a recombinant protein and immobilized on a sensor. The sensor was contacted with a solution comprising LRRC15-Fc (500 nM) or EVs comprising LRRC15 full-length or gD-GPI LRRC15 (0.25 mg/ml), and the BLI signal (in nm) was measured.

[0161] FIG. **11**B is a schematic diagram and a set of micrographs showing binding of EVs comprising gag-NeonGreen and full-length (FL) LRRC15, gD-GPI LRRC15 (LRRC15-gD), or an empty-vector control to cells transiently expressing full-length CD248 or gD-GPI CD248.

[0162] FIG. **11**C is a schematic diagram and a set of micrographs showing binding of CD248 (as a tetramerized recombinant ectodomain) or an empty-vector control to cells transiently expressing full-length LRRC15 or gD-GPI LRRC15. Scale bar represents 20 μm.

[0163] FIG. **11**D is a bar graph showing a quantification of EV binding in FIG. **11**B based on NeonGreen signal levels (mean±standard error for three independent replicates).

[0164] FIG. **12**A is a scatter plot showing a comparison of *Renilla* luciferase fluorescence and fluorescence of cholesterol detected using the AMPLEX™ Red Cholesterol Assay Kit (Thermo Fisher) (Cholesterol) as a readout for a dilution series of PD-L1 and PVR gD-GPI EVs harvested from EXPI293F™ cells transfected with gag-Rluc or untransfected cells.

[0165] FIG. **12**B is a bar graph showing the relative signal levels from *Renilla* luciferase and the AMPLEX™ Red Cholesterol Assay Kit (Thermo Fisher) observed in a small-scale RDIMIS screen with the listed genes immobilized in wells and probed using PD-L1 gD-GPI EVs. Results for both readouts are normalized to the respective PDCD1 signals.

[0166] FIG. **12**C is a scatter plot showing the results of an RDIMIS screen performed using cholesterol as a readout on EVs expressing gag-Rluc (x-axis) or vesicles harvested from untransfected cells (y-axis).

[0167] FIG. **13**A is a set of photomicrographs showing Western blots of EVs expressing full-length (FL) or gD-GPI tagged ectodomains of the receptors PVR, PD-L1, CD276, CD80, and LRRC15 stained with an antibody specific for the gD tag (α-gD). α-Actin, α-PVR, and α-LRRC15 staining are also shown.

[0168] FIG. **13**B is a schematic diagram and a graph showing the design and results of a BLI experiment. Biotinylated PD-L1 was incubated with a streptavidin BLI biosensor. This was then incubated with EVs expressing full-length or gD-GPI LRTM1 or a vector control, and the BLI signal (in nm) was measured.

[0169] FIG. **13**C is a set of micrographs showing EVs bound to the surface of cells transiently expressing full-length or gD-GPI tagged PD-L1. EVs contained full-length or gD-GPI LRTM1 or a vector control. EVs comprised gag-NeonGreen, and green represents direct fluorescence from the EVs. DNA of the mammalian cells is shown in blue.

[0170] FIG. **13**D is a schematic diagram and a graph showing the design and results of a BLI experiment. Biotinylated PD-L1 was incubated with a streptavidin BLI biosensor. This was then incubated with EVs expressing full-length or gD-GPI LRTM1 or a vector control in the presence or absence of different concentrations of Fc-tagged PD1 ectodomain or a human IgG control, and the BLI signal (in nm) was measured.

[0171] FIG. **14**A is a scatter plot showing the results of an RDIMIS screen performed using EVs comprising PVR gD-GPI (Y axis) compared to "empty" EVs derived from cells transfected with a vector control (X axis).

[0172] FIG. **14**B is a scatter plot showing the results of an RDIMIS screen performed using EVs comprising PD-L1 gD-GPI (Y axis) compared to "empty" EVs derived from cells transfected with a vector control (X axis).

[0173] FIG. **14**C is a scatter plot showing the results of an RDIMIS screen performed using EVs comprising CD80 gD-GPI (Y axis) compared to "empty" EVs derived from cells transfected with a vector control (X axis).

[0174] FIG. **14**D is a scatter plot showing the results of an RDIMIS screen performed using EVs comprising CD276 gD-GPI (Y axis) compared to "empty" EVs derived from cells transfected with a vector control (X axis).

[0175] FIG. **14**E is a scatter plot showing the results of an RDIMIS screen performed using EVs comprising LRRC15 gD-GPI (Y axis) compared to "empty" EVs derived from cells transfected with a vector control (X axis).

[0176] FIG. **14**F is a scatter plot showing the results of an RDIMIS screen performed using EVs comprising full-length LRRC15 (Y axis) compared to "empty" EVs derived from cells transfected with a vector control (X axis).

[0177] FIG. **15** is a network diagram showing the generic vesicle binders identified herein (green boxes) integrated with the IgSF Interactome's list of high confidence interactions (1) (blue edges) and the experimental and database list of interactions from STRING (2) (red edges) to identify potential interaction partners (blue boxes). The height of the

boxes represent normalized expression values in HEK293 cells from The Cell Atlas (3) to estimate the expression of the potential binding partners in the EV parent cells and, therefore, the EVs themselves.

[0178] FIG. **16**A is a set of scatter plots showing correlations and correlation coefficients for each of the RDIMIS screens performed. Screens were done in several batches: 1) PVR gD-GPI repeat 1, 2) PVR gD-GPI repeat 2 and PD-L1 gD-GPI, 3) CD80 gD-GPI and CD26 gD-GPI, 4) LRRC15 gD-GPI, LRRC15 FL and PVR FL, 5) Vesicle control which are EVs with no overexpressed receptor-of-interest.

[0179] FIG. **16**B is a set of scatter plots showing the correlation between CD80 gD-GPI and PVR-FL screens. Two populations of generic vesicle binders are shown. Lower panel: zoomed-in view of x axis.

[0180] Right panel: correlation with generic vesicle binders removed.

[0181] FIG. **17**A is a graph showing the results of a BLI experiment in which membranes were disrupted with the cholesterol binder Filipin III. CD248 monomer were loaded onto a NiNTA biosensor and incubated with LRRC15 gD-GPI EVs that had been pre-treated for 30 minutes at room temperature with filipin III. Empty vesicles or filipin III are shown as negative controls.

[0182] FIG. **17**B is a graph showing the results of a BLI experiment in which membranes were disrupted with the cholesterol binder Filipin III. CD248 monomer were loaded onto a NiNTA biosensor and incubated with full-length LRRC15 EVs that had been pre-treated for 30 minutes at room temperature with filipin III. Empty vesicles or filipin III are shown as negative controls.

[0183] FIG. **17**C is a graph showing binding of the EVs of FIG. **17**A to an anti-gD antibody.

[0184] FIG. **17**D is a graph showing the results of a BLI experiment in which membranes were disrupted with Methyl-beta cyclodextrin (MpβCD). CD248 monomer were loaded onto a NiNTA biosensor and incubated with LRRC15 gD-GPI EVs that had been pre-treated for 30 minutes at room temperature with filipin III. Empty vesicles or filipin III are shown as negative controls.

[0185] FIG. **17**E is a graph showing the results of a BLI experiment in which membranes were disrupted with MpβCD. CD248 monomer were loaded onto a NiNTA biosensor and incubated with full-length LRRC15 EVs that had been pre-treated for 30 minutes at room temperature with filipin III. Empty vesicles or filipin III are shown as negative controls.

[0186] FIG. **17**F is a graph showing binding of the EVs of FIG. **17**D to an anti-gD antibody.

[0187] FIG. **18**A is a scatter plot showing level of antibody surface staining (a.u.) for >500 multi-transmembrane receptors expressed on cells and a pair of photomicrographs showing representative cell surface staining for a low-expressing receptor (DRD2) and a high-expressing receptor (S1 PR1). Background staining is denoted by the line.

[0188] FIG. **18**B is a scatter plot showing level of surface staining (a.u.) using an anti-FLAG antibody and fluorescence of a Venus tag (X-axis; total receptor (a.u.)) for >400 G protein-coupled receptors (GPCRs) engineered with an N-terminal FLAG tag and a C-terminal Venus. The inset images are photomicrographs showing representative cell surface staining (magenta) and Venus fluorescence (green) for a low-expressing receptor (DRD2), a high-expressing receptor (S1 PR1), and a very highly expressed single-transmembrane receptor (EGFR). Background staining was determined by average of signal on untransfected cells and is denoted by the line.

[0189] FIG. **18**C is a circle chart showing characteristics of the 1791 members of the multi-transmembrane (MTMR) receptor library. Only >500 members have an extracellular HIS tag, and only about half of those receptors show staining above background.

[0190] FIG. **18**D is a circle chart showing the proportions of the GPCRs of FIG. **18**B having low, medium, and high FLAG staining expression levels. "Medium" receptor expression was defined as ten times the background signal.

[0191] FIG. **19**A is a scatter plot showing results of a screen for binding (a.u.) of EGF-647 to members of the multi-transmembrane receptor library. EGF-647 bound only to EGFR, which was printed on each plate as a transfection control. The inset panel is a photomicrograph showing the fluorescent ligand. DAPI staining is shown.

[0192] FIG. **19**B is a scatter plot showing results of a screen for binding (a.u.) of RSPO3 to members of the multi-transmembrane receptor library. RSPO3 bound to LGR4 and LGR5. Imaging artifacts are denoted by an X. The inset panel is a photomicrograph showing the fluorescent ligand. DAPI staining is shown.

[0193] FIG. **19**C is a scatter plot showing results of a screen for binding (a.u.) of PVR to members of the multi-transmembrane receptor library. PVR bound to CD226, a single-pass transmembrane receptor that was added as a positive control. The inset panel is a photomicrograph showing the fluorescent ligand. DAPI staining is shown.

[0194] FIG. **19**D is a scatter plot showing results of a screen for binding (a.u.) of PD-L1 to members of the multi-transmembrane receptor library. PD-L1 bound to PVR the adhesion G protein-coupled receptor B1 (ADGRB1), as well as to the single-pass transmembrane receptors PD1, PDL2, CD80, and EPHA3, which were added as positive controls. The inset panel is a photomicrograph showing the fluorescent ligand. DAPI staining is shown.

[0195] FIG. **20**A is a schematic diagram showing an extracellular vesicle (EV) comprising a tagged multi-pass transmembrane receptor. The extracellular regions of the receptor are on the outside of the EV, and the intracellular regions are in the lumen of the EV. Locations of the FLAG tag and fluorescent tag (Luc) are shown.

[0196] FIG. **20**B is a negative stain electron microscopy image showing EVs.

[0197] FIG. **20**C is a graph showing the size distribution (in nm) and concentration (10^6 particles per mL) of EVs from cells transfected with PVR and GAG; transfected with PVR only; or not transfected (control), as measured using NanoSight particle tracking.

[0198] FIG. **20**D is a bar graph showing the mean size (nm) of the EVs of FIG. **20**C.

[0199] FIG. **20**E is a graph showing the results of a BLI experiment assessing binding of an anti-gD antibody to EVs derived from cells transfected with PVR and GAG; PVR only; or EVs from untransfected cells.

[0200] FIG. **20**F is a graph showing the results of a BLI experiment assessing binding of the PVR ligand TIGIT (TIGIT Fc) to EVs derived from cells transfected with PVR and GAG; PVR only; or EVs from untransfected cells.

[0201] FIG. **20**G is a graph showing the results of a BLI experiment assessing binding of an anti-FLAG antibody to

EVs comprising the G protein-coupled receptors (GPCRs) ADGRB1, LGR4, and LGR5. GPCRs comprised an N-terminal FLAG tag.

[0202] FIG. **21**A is a scatter plot showing results of a screen for binding (a.u.) of EVs comprising PVR to members of the multi-transmembrane receptor library and to positive controls. PVR bound to the positive controls. Imaging artifacts are denoted by an X. The inset panel is a photomicrograph showing vesicle fluorescence from GAG-neonGreen fusion. DAPI staining is shown.

[0203] FIG. **21**B is a scatter plot showing results of a screen for binding (a.u.) of EVs comprising PD-L1 to members of the multi-transmembrane receptor library and to positive controls. PVR bound to the positive controls and to ADGRB1. Imaging artifacts are denoted by an X. The inset panel is a photomicrograph showing vesicle fluorescence from GAG-neonGreen fusion. DAPI staining is shown.

[0204] FIG. **21**C is a scatter plot showing results of a screen for binding (a.u.) of ADGRB1 to members of a library comprising the extracellular domains of Fc-fused single transmembrane receptors (STMRs). Interactions with RTN4R and PD-L1 were confirmed and new interactions were revealed.

[0205] FIG. **22**A is a bar graph showing quantification of binding of the recombinant proteins PD-L1-Fc, ICOSLG-Fc, and RTN4R-Fc (each conjugated to a protein A plate) to EVs comprising ADGRB1 or LGR4.

[0206] FIG. **22**B is a set of photomicrographs showing the results of assays for binding of the recombinant proteins PD-L1-Fc, ICOSLG-Fc, and RTN4R-Fc to HEK cells expressing ADGRB1 fused to Venus. In the merged image, DAPI is shown in blue; the Venus signal from the ADGRB1 fusion protein is shown in green, and the signal for staining of the Fc tag is shown in magenta. Co-localized green and magenta signals are shown in white.

[0207] FIG. **23** is a schematic diagram showing the design of the GPCR screening platform. Comprehensive libraries are collected in 384-well plate format. A comprehensive collection of overexpression plasmids are printed onto 384-well imaging plates. Cells are reverse transfected, then treated with fluorescent ligand and analyzed in a high throughput, high content imager FIG. **24**A is a set of photomicrographs showing the results of assays for binding of the recombinant protein PD-L1-Fc to HEK cells expressing ADGRB1 fused to Venus. In the merged image, DAPI is shown in blue; the Venus signal from the ADGRB1 fusion protein is shown in green, and the signal for staining of the Fc tag is shown in magenta. Co-localized green and magenta signals are shown in white. All contrast and brightness settings are matched to FIG. **22**B.

[0208] FIG. **24**B is a set of photomicrographs showing the results of assays for binding of the recombinant protein ICOSLG-Fc to HEK cells expressing ADGRB1 fused to Venus. In the merged image, DAPI is shown in blue; the Venus signal from the ADGRB1 fusion protein is shown in green, and the signal for staining of the Fc tag is shown in magenta. Co-localized green and magenta signals are shown in white. All contrast and brightness settings are matched to FIG. **22**B.

[0209] FIG. **24**C is a set of photomicrographs showing the results of assays for binding of the recombinant protein RTN4R-Fc to HEK cells expressing ADGRB1 fused to Venus. In the merged image, DAPI is shown in blue; the Venus signal from the ADGRB1 fusion protein is shown in green, and the signal for staining of the Fc tag is shown in magenta. Co-localized green and magenta signals are shown in white. All contrast and brightness settings are matched to FIG. **22**B.

[0210] FIG. **25**A is a graph showing the results of bioluminescent energy transfer (BRET) assays for β-arrestin and SH2 recruitment in HEK cells. No activation of ADGRB1 or EphA3 was observed in response to PD-L1.

[0211] FIG. **25**B is a graph showing calcium sensing (GCaMP6s fluorescence) following treatment of HEK cells. No response was observed downstream of ADGRB1 or EphA3 in response to PD-L1.

[0212] FIG. **25**C is a graph showing cAMP stimulation (assessed by GLOSENSOR™) following treatment of HEK cells. No response was observed.

[0213] FIG. **25**D is a graph showing cAMP inhibition following treatment of HEK cells. No response was observed.

[0214] FIG. **26**A is a scatter plot showing results of a screen for binding of EVs comprising ADGRB1 to members of a secreted protein Fc library. A positive control (anti-FLAG antibody) is labeled.

[0215] FIG. **26**B is a scatter plot showing results of a screen for binding of EVs comprising ADGRB1 to members of a library comprising the extracellular domains of single transmembrane receptors fused to Fc (STM library). Binding to RTN4R and PD-L1 was confirmed, and new interactions were identified. Novel hits are labeled.

[0216] FIG. **26**C is a scatter plot showing results of a screen for binding of EVs comprising LGR4 to members of a secreted protein Fc library. A positive control (anti-FLAG antibody) is labeled.

[0217] FIG. **26**D is a scatter plot showing results of a screen for binding of EVs comprising LGR4 to members of the STM library. A positive control (RSPO3) is labeled. Novel hits are shown in green.

[0218] FIG. **26**E is a scatter plot showing results of a screen for binding of EVs comprising LGR5 to members of a secreted protein Fc library. A positive control (anti-FLAG antibody) is labeled. Novel hits are shown in green.

[0219] FIG. **26**F is a scatter plot showing results of a screen for binding of EVs comprising LGR5 to members of the STM library. A positive control (anti-FLAG antibody) is labeled. Novel hits are shown in green. Shared LGR4 and LGR5 hits are shown in blue.

[0220] FIG. **27** is a bar graph showing the results of a carboxypeptidase M (CPM) activity assay. CPM-FL: vesicles comprised full-length CPM. CPMgD: vesicles comprised gD-GPI (gD) CPM. pRK EV: vector control; PBS only: buffer control.

[0221] FIG. **28** is a pair of photomicrographs showing a pair of Western blots showing levels of total EPHA3 and phosphorylated EPHA3 species (pEPHA2/3/4 and pEPHA3/4/5) detected in EVs comprising full-length EPHA3 (EPHA3-FL) and PDL1-Fc, EPHA3-Fc, the EPHA3 ligands EFNA1-Fc and EFNA5-Fc, and full-length PDL1. pRK EV: vector control.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

[0222] As used herein, the term "biological vesicle" or "By" refers to a lipid bilayer-delimited particle that is

naturally secreted from a parent cell, e.g., a mammalian cell. BVs may be, e.g., extracellular vesicles (EVs; nanometer-sized particles, e.g., recombinant extracellular vesicles (rEVs)), exosomes, microvesicles, or virus-like particles (VLPs). VLPs are described, e.g., in Titeca et al., *Nature* Protocols, 12(5): 881-898, 2017). BV compositions or preparations may include only one of EVs, exosomes, microvesicles, or VLPs, or may include a mixture of two, three, or all four of EVs, exosomes, microvesicles, and VLPs. BVs contain proteins folded and inserted into their native membranes using the parent cell's endogenous machinery. In some aspects, BVs include proteins that are not native to the parent cell, e.g., proteins that the parent cell has been modified to express (e.g., heterologous membrane-associated proteins, e.g., heterologous membrane-associated proteins comprising a protein fragment, a tag, and an anchor). Production of BVs by a parent cell may be increased by contacting the parent cell with a membrane-budding agent (e.g., transforming the cell with a membrane-budding agent, e.g., a HIV gag protein) and/or exposing the cell to conditions that promote the formation of BVs. In some aspects, the BV is purified from the parent cell (e.g., purified from a culture medium comprising the parent cell).

[0223] As used herein, the term "membrane-budding agent" refers to an agent that increases the production of BVs (e.g., extracellular vesicles (EVs), exosomes, microvesicles, and/or virus-like particles (VLPs)) by the parent cell. In some aspects, the membrane-budding agent is an HIV gag protein. In some aspects, the HIV gag protein has the amino acid sequence of SEQ ID NO: 1. In some aspects, the HIV gag protein has at least 90% identity to the amino acid sequence of SEQ ID NO: 1, e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 1. In some aspects, the parent cells are transformed with the membrane-budding agent. Cells may additionally or alternatively be exposed to a condition (e.g., a culture condition) that increases the production of BVs (e.g., extracellular vesicles (EVs), exosomes, microvesicles, and/or virus-like particles (VLPs)) by the parent cell. Further examples of membrane-budding agents include self-assembling VLPs (e.g., MLGag, AARDC1 (e.g., hAARDC1), and Acyl.Hrs); agents that enhance endogenous vesicle formation pathways such as exosome or tumor pathways (e.g., RhoA.F3OL, ARF6.Q67L, VPS4a, HAS3, CD9, CD63, and CD81); and factors associated with apoptotic bodies (e.g., constitutively active ROCK1).

[0224] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) aspects that are directed to that value or parameter per se.

[0225] The term "single transmembrane receptor," "single-pass transmembrane receptor," or "STM receptor," as used herein, refers to a protein having a single transmembrane domain. In some aspects, the STM receptor is expressed on the cell surface. Exemplary STM receptors are provided in Table 4, as well as in PCT/US2020/025471, Martinez-Martin et al., *Cell,* 174(5): 1158-1171, 2018, and Clark et al., *Genome Res,* 13: 2265-2270, 2003, each of which is incorporated by reference herein in its entirety. In some aspects, the STM protein has the UniProt annotation "leucine-rich," "cysteine-rich," "ITIM/ITAM" (immunoreceptor tyrosine-based inhibition motif/immunoreceptor tyrosine-based activation motif), "TNFR" (tumor necrosis factor receptor), "TLR/ILR" (Toll-like receptor/interleukin receptor), "semaphorin," "Kinase-like," "Ig-like" (immunoglobulin-like), "fibronectin," "ephrin," "EGF," "cytokineR," or "cadherin." STM receptors may be identified based on, e.g., the presence of a signal peptide or a predicted transmembrane region in the amino acid sequence. In some aspects, the STM receptor is expressed as an extracellular domain.

[0226] As used herein, the term "extracellular domain" or "ECD" refers to a protein domain that is predicted to be localized outside of the outer plasma membrane of the cell. In some instances, the ECD is an ECD of a receptor, e.g., a STM receptor. In some aspects, the ECD is an ECD of an IgSF protein. In some aspects, the ECD is the ECD of PDPN. In some aspects, the boundaries of the extracellular domain may be identified by prediction of domains that indicate that the protein crosses the plasma membrane, e.g., a transmembrane domain (e.g., a transmembrane helix). In some aspects, the presence of an extracellular domain may be predicted by the presence of a domain, sequence, or motif that indicates that the protein is trafficked to the plasma membrane, e.g., a signal sequence or a glycosylphosphatidylinositol (GPI) linkage site. In some aspects, the boundaries of the ECD are determined according to UniProt annotations. In some aspects, the ECD is soluble. In some aspects, the extracellular domain is expressed in the context of a full-length protein. In other aspects, the extracellular domain is expressed as an isolated extracellular domain, e.g., a sequence of amino acid residues comprising only the amino acid residues of a protein that are predicted to be extracellular.

[0227] In some aspects, the isolated ECD is included in a fusion protein. In some aspects, inclusion in a fusion protein increases solubility, ease of expression, ease of capture (e.g., on a protein A-coated plate), multimerization, or some other desirable property of the ECD. In some aspects, the ECD or ECD fusion protein is a monomer. In other aspects, the ECD or ECD fusion protein is a multimer, e.g., a tetramer or a pentamer. In some aspects, the ECD is fused to a human IgG. In some aspects, the ECD is fused to a human Fc tag. In some aspects, the ECD is fused to an Avidity AVITAG™ (Avi tag). In some aspects, the ECD is fused to a polyhistidine (His) tag. In some aspects, the ECD is fused to a glycoprotein D (gD) tag and a glycosylphosphatidylinositol (GPI) linker, e.g., a gD-GPI tag. In other aspects, the ECD is fused to the pentamerization domain of rat cartilaginous oligomeric matrix protein (COMP) and the β-lactamase protein, e.g., as described in Bushell et al., *Genome Res,* 18: 622-630, 2008. In some aspects, the ECD fusion protein further includes a cleavage sequence, e.g., a TEV cleavage sequence, to allow removal of one or more domains. In some instances, an ECD fusion protein having an Avi tag and an Fc tag cleavable at a cleavage sequence is further processed to remove the Fc tag, to biotinylate the Avi tag, and to fuse the biotinylated ECD fusion protein to a fluorescent streptavidin (SA), e.g., to form a tetramerized ECD fusion protein. In some instances, the isolated ECD or ECD fusion protein is purified.

[0228] As used herein, a "modulator" is an agent that modulates (e.g., increases, decreases, activates, or inhibits) a given biological activity, e.g., an interaction or a downstream activity resulting from an interaction. A modulator or candidate modulator may be, e.g., a small molecule, an antibody, an antigen-binding fragment (e.g., a bis-Fab, an

Fv, a Fab, a Fab'-SH, a F(ab')$_2$, a diabody, a linear antibody, an scFv, an ScFab, a VH domain, or a VHH domain), a peptide, a mimic, an antisense oligonucleotide (ASO), or a small interfering RNA (siRNA).

[0229] By "increase" or "activate" is meant the ability to cause an overall increase, for example, of 20% or greater, of 50% or greater, or of 75%, 85%, 90%, or 95% or greater. In certain aspects, increase or activate can refer to a downstream activity of a protein-protein interaction.

[0230] By "reduce" or "inhibit" is meant the ability to cause an overall decrease, for example, of 20% or greater, of 50% or greater, or of 75%, 85%, 90%, or 95% or greater. In certain aspects, reduce or inhibit can refer to a downstream activity of a protein-protein interaction.

[0231] "Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., a receptor) and its binding partner (e.g., a ligand). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity, which reflects a 1:1 interaction between members of a binding pair (e.g., receptor and ligand). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured by common methods known in the art, including those described herein.

[0232] "Complex" or "complexed" as used herein refers to the association of two or more molecules that interact with each other through bonds and/or forces (e.g., Van der Waals, hydrophobic, hydrophilic forces) that are not peptide bonds. In one aspect, a complex is heteromultimeric. It should be understood that the term "protein complex" or "polypeptide complex" as used herein includes complexes that have a non-protein entity conjugated to a protein in the protein complex (e.g., including, but not limited to, chemical molecules such as a toxin or a detection agent).

[0233] The term "parent cell" refers to cells from which BVs are produced. Parent cells include cells into which an exogenous nucleic acid has been introduced, including the progeny of such cells. Parent cells include "transfected cells," "transformed cells," and "transformants," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein. In some aspects, the parent cell is stably transformed with the exogenous nucleic acid. In other aspects, the parent cell is transiently transformed with the exogenous nucleic acid.

[0234] The term "leucine-rich repeat-containing protein 15" or "LRRC15," as used herein, broadly refers to any native LRRC15 from any mammalian source, including primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses full-length LRRC15 and isolated regions or domains of LRRC15, e.g., the LRRC15 ECD. The term also encompasses naturally occurring variants of LRRC15, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human LRRC15 is shown under UniProt Accession No. Q8TF66. Minor sequence variations, especially conservative amino acid substitutions of LRRC15 that do not affect LRRC15 function and/or activity, are also contemplated by the invention.

[0235] The term "programmed cell death 1 ligand 1" or "PD-L1," as used herein, broadly refers to any native PD-L1 from any mammalian source, including primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. PD-L1 is also called CD274. The term encompasses full-length PD-L1 and isolated regions or domains of PD-L1, e.g., the PD-L1 ECD. The term also encompasses naturally occurring variants of PD-L1, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human PD-L1 is shown under UniProt Accession No. Q9NZQ7. Minor sequence variations, especially conservative amino acid substitutions of PD-L1 that do not affect PD-L1 function and/or activity, are also contemplated by the invention.

[0236] The term "poliovirus receptor" or "PVR," as used herein, broadly refers to any native PVR from any mammalian source, including primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses full-length PVR and isolated regions or domains of PVR, e.g., the PVR ECD. The term also encompasses naturally occurring variants of PVR, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human PVR is shown under UniProt Accession No. A0A0C4DG49. Minor sequence variations, especially conservative amino acid substitutions of PVR that do not affect PVR function and/or activity, are also contemplated by the invention.

[0237] The term "CD80," as used herein, broadly refers to any native CD80 from any mammalian source, including primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. CD80 is also called B7-1. The term encompasses full-length CD80 and isolated regions or domains of CD80, e.g., the CD80 ECD. The term also encompasses naturally occurring variants of CD80, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human CD80 is shown under UniProt Accession No. P33681. Minor sequence variations, especially conservative amino acid substitutions of CD80 that do not affect CD80 function and/or activity, are also contemplated by the invention.

[0238] The term "CD276," as used herein, broadly refers to any native CD276 from any mammalian source, including primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. CD276 is also called B7-H3. The term encompasses full-length CD276 and isolated regions or domains of CD276, e.g., the CD276 ECD. The term also encompasses naturally occurring variants of CD276, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human CD276 is shown under UniProt Accession No. Q5ZPR3. Minor sequence variations, especially conservative amino acid substitutions of CD276 that do not affect CD276 function and/or activity, are also contemplated by the invention.

[0239] The terms "TEM1," "CD248," and "endosialin," as used herein, broadly refer to any native TEM1 from any mammalian source, including primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses full-length TEM1 and isolated regions or domains of TEM1, e.g., the TEM1 ECD. The term also encompasses naturally occurring variants of TEM1, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human TEM1 is shown under UniProt Accession No. Q9HCU0. Minor sequence variations, espe-

cially conservative amino acid substitutions of TEM1 that do not affect TEM1 function and/or activity, are also contemplated by the invention.

[0240] The terms "ADGRB1," "adhesion GPCR B11," and "adhesion G protein-coupled receptor B11," as used herein, broadly refer to any native ADGRB1 from any mammalian source, including primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses full-length ADGRB1 and isolated regions or domains of ADGRB1, e.g., the ADGRB1 ECDs. The term also encompasses naturally occurring variants of ADGRB1, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human ADGRB1 is shown under UniProt Accession No. O14514. Minor sequence variations, especially conservative amino acid substitutions of TEM1 that do not affect TEM1 function and/or activity, are also contemplated by the invention.

[0241] The terms "ICOSLG," "inducible T cell costimulatory ligand," and "ICOS ligand," as used herein, broadly refer to any native ICOSLG from any mammalian source, including primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses full-length ICOSLG and isolated regions or domains of ICOSLG, e.g., the ICOSLG ECD. The term also encompasses naturally occurring variants of ICOSLG, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human ICOSLG is shown under UniProt Accession No. 075144. Minor sequence variations, especially conservative amino acid substitutions of ICOSLG that do not affect ICOSLG function and/or activity, are also contemplated by the invention.

[0242] The term "protein," as used herein, refers to any native protein from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed protein any form of the protein that results from processing in the cell. The term also encompasses naturally occurring variants of the protein, e.g., splice variants or allelic variants, e.g., amino acid substitution mutations or amino acid deletion mutations. The term also includes isolated regions or domains of the protein, e.g., the extracellular domain (ECD).

[0243] An "isolated" protein or peptide is one which has been separated from a component of its natural environment. In some aspects, a protein or peptide is purified to greater than 95% or 99% purity as determined by, for example, electrophoresis (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatography (e.g., ion exchange or reverse phase HPLC).

[0244] An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[0245] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

[0246] The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments (e.g., bis-Fabs) so long as they exhibit the desired antigen-binding activity.

[0247] An "antigen-binding fragment" or "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antigen-binding fragments include but are not limited to bis-Fabs; Fv; Fab; Fab, Fab'-SH; $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g., scFv, ScFab); and multispecific antibodies formed from antibody fragments.

[0248] A "single-domain antibody" refers to an antibody fragment comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain aspects, a single-domain antibody is a human single-domain antibody (see, e.g., U.S. Pat. No. 6,248,516 B1). Examples of single-domain antibodies include but are not limited to a VHH.

[0249] A "Fab" fragment is an antigen-binding fragment generated by papain digestion of antibodies and consists of an entire L chain along with the variable region domain of the H chain (VH), and the first constant domain of one heavy chain (CH1). Papain digestion of antibodies produces two identical Fab fragments. Pepsin treatment of an antibody yields a single large $F(ab')_2$ fragment which roughly corresponds to two disulfide linked Fab fragments having divalent antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having an additional few residues at the carboxy terminus of the CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0250] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all Lys447 residues removed, antibody populations with no Lys447 residues removed, and antibody populations having a mixture of antibodies with and without the Lys447 residue.

[0251] "Fv" consists of a dimer of one heavy- and one light-chain variable region domain in tight, noncovalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L

chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although often at a lower affinity than the entire binding site.

[0252] The terms "full-length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

[0253] "Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the VH and VL antibody domains connected into a single polypeptide chain. Preferably, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains, which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see Pluckthun, The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); Malmborg et al., J. Immunol. Methods 183:7-13, 1995.

[0254] The term "small molecule" refers to any molecule with a molecular weight of about 2000 daltons or less, e.g., about 1000 daltons or less. In some aspects, the small molecule is a small organic molecule.

[0255] The term "mimic," "peptide mimic," "polypeptide mimic," or "molecular mimic," as used herein, refers to a polypeptide having sufficient similarity in conformation and/or binding ability (e.g., secondary structure, tertiary structure) to a given polypeptide or to a portion of said polypeptide to bind to a binding partner of said polypeptide. The mimic may bind the binding partner with equal, less, or greater affinity than the polypeptide it mimics. A molecular mimic may or may not have obvious amino acid sequence similarity to the polypeptide it mimics. A mimic may be naturally occurring or may be engineered. In some aspects, the mimic is a mimic of the protein of Table 1. In other aspects, the mimic is a mimic of the protein of Table 2. In yet other aspects, the mimic is a mimic of another protein that binds to the protein of Table 1 or the protein of Table 2. In some aspects, the mimic is a mimic of the protein of Table 5. In other aspects, the mimic is a mimic of the protein of Table 6. In yet other aspects, the mimic is a mimic of another protein that binds to the protein of Table 5 or the protein of Table 6. In some aspects, the mimic may perform all functions of the mimicked polypeptide. In other aspects, the mimic does not perform all functions of the mimicked polypeptide.

[0256] As used herein, the term "conditions permitting the binding" of two or more proteins to each other (e.g., a protein of Table 1 and a protein of Table 2 or a protein of Table 5 and a protein of Table 6) refers to conditions (e.g., protein concentration, temperature, pH, salt concentration) under which the two or more proteins would interact in the absence of a modulator or a candidate modulator. Conditions permitting binding may differ for individual proteins and may differ between protein-protein interaction assays (e.g., surface plasmon resonance assays, biolayer interferometry assays, enzyme-linked immunosorbent assays (ELISA), extracellular interaction assays, and cell surface interaction assays.

[0257] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0258] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0259] The term "sample," as used herein, refers to a composition that is obtained or derived from a subject and/or individual of interest that contains a cellular and/or other molecular entity that is to be characterized and/or identified, for example, based on physical, biochemical, chemical, and/or physiological characteristics. For example, the phrase "disease sample" and variations thereof refers to any sample obtained from a subject of interest that would be expected or is known to contain the cellular and/or molecular entity that is to be characterized. Samples include, but are not limited to, tissue samples, primary or cultured cells or cell lines, cell supernatants, cell lysates, platelets, serum, plasma, vitreous fluid, lymph fluid, synovial fluid, follicular fluid, seminal fluid, amniotic fluid, milk, whole blood, plasma, serum,

blood-derived cells, urine, cerebro-spinal fluid, saliva, buccal swab, sputum, tears, perspiration, mucus, tumor lysates, and tissue culture medium, tissue extracts such as homogenized tissue, tumor tissue, cellular extracts, and combinations thereof. The sample may be an archival sample, a fresh sample, or a frozen sample. In some aspects, the sample is a formalin-fixed and paraffin-embedded (FFPE) tumor tissue sample.

II. Biological Vesicles Displaying Proteins

[0260] The study of interactions involving membrane-bound proteins (e.g., receptor-ligand interactions) is instrumental to understanding cellular communication occurring in the extracellular milieu. However, progress on identifying and understanding these interactions has lagged behind that of cytoplasmic proteins, in part because receptor-ligand interactions take place in membranes. Physiological membranes contain a complex mix of lipids, sterols, proteins and glycans, all of which can participate in interactions. In addition, membranes help cluster, orient and fold receptors, strengthening weak protein-protein interactions. Standard methods for assessing protein-protein interactions typically require the absence of, or extraction from, cellular membranes. As a result, these commonly utilized methodologies underrepresent receptor-ligand interactions.

[0261] The disclosure features proteins (e.g., transmembrane receptors) displayed on the surface of biological vesicles (BVs), e.g., extracellular vesicles (EVs). In some aspects, the disclosure features a BV comprising (a) a heterologous membrane-associated protein comprising a protein fragment, a tag, and an anchor, wherein the heterologous membrane-associated protein is present on the outer face of the BV and (b) a membrane-budding agent. In some aspects, the membrane-budding agent is an HIV gag protein.

[0262] In some aspects, the disclosure features a BV comprising (a) a heterologous membrane-associated protein comprising a protein fragment, a tag, and an anchor, wherein the heterologous membrane-associated protein is present on the outer face of the BV and (b) a membrane-budding agent, wherein the membrane budding agent is an HIV gag protein, the BV being produced by a process comprising (i) providing a parent cell that has been modified to express the heterologous membrane-associated protein and the membrane-budding agent and (ii) isolating the BV from the parent cell.

[0263] A. Protein Fragments

[0264] In some aspects, a protein fragment is an extracellular domain of a transmembrane receptor, e.g., a single-pass transmembrane (STM) receptor or a multi-pass transmembrane receptor (multi-transmembrane receptor; MTMR), e.g., a G protein-coupled receptor (GPCR). Exemplary STM receptors are described in Section III herein and are provided in Table 2 and Table 4.

[0265] B. Anchors

[0266] In some aspects, an anchor tethers the protein fragment to the surface of a lipid membrane of a BV. In some aspects, the anchor is a glycosylphosphatidyl-inositol (GPI) polypeptide. In some aspects, the anchor is a moiety used in protein lipidation, e.g., a moiety used in cysteine palmitoylation, glycine myristoylation, lysine fatty-acylation, cholesterol esterification, cysteine prenylation, or serine fatty-acylation.

[0267] C. Tags

[0268] In some aspects, a tag can be directly or indirectly visualized, or otherwise detected. For example, the tag may comprise a moiety that can be detected using an antibody or an antibody fragment, e.g., may be a glycoprotein D (gD) polypeptide. In some aspects, the tag comprises a fluorescent protein. In some aspects, the protein fragment is conjugated (e.g., fused) to a gD-GPI construct comprising a gD tag and a GPI anchor.

[0269] D. Membrane-Budding Agents

[0270] BVs may further comprise a membrane-budding agent that, when present in the parent cell, increases the production of BVs (e.g., extracellular vesicles (EVs), exosomes, microvesicles, and/or virus-like particles (VLPs)) by the parent cell. The parent cell may be transfected with the membrane-budding agent, and the membrane-budding agent may be inherited by the BV, e.g., during the membrane budding process.

[0271] In some aspects, the membrane-budding agent is an HIV gag protein. In some aspects, the HIV gag protein has the amino acid sequence of SEQ ID NO: 1. In some aspects, the HIV gag protein has at least 90% identity to the amino acid sequence of SEQ ID NO: 1, e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 1. In some aspects, the membrane-budding agent (e.g., HIV gag protein) comprises a marker that can be directly or indirectly visualized, or otherwise detected. In some aspects, the detectable marker is a fluorescent protein. In some aspects, the detectable marker is an enzyme that produces a fluorescent signal in the presence of a substrate, e.g., *Renilla* luciferase (Rluc). Additional membrane-budding agents are described in Section III(C), below.

[0272] Cells may additionally or alternatively be exposed to a condition (e.g., a culture condition) that increases the production of BVs (e.g., extracellular vesicles (EVs), exosomes, microvesicles, and/or virus-like particles (VLPs)) by the parent cell. EVs comprising a membrane-budding agent may be referred to as recombinant EVs (rEVs).

[0273] The membrane-budding agent and/or agent or condition that increases BV production may increase BV production by the parent cell by, e.g., 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, or more than 4-fold (e.g., 1.5 to 2.5-fold, 2.5 to 3.5-fold, or 3.5 to 4.5-fold). In some aspects, the membrane-budding agent (e.g., HIV gag protein) increases BV production by the parent cell by about 4-fold.

[0274] E. Parent Cells and Methods of Isolation

[0275] Biological vesicles (BVs) include any suitable lipid vesicle structure that has been derived from (e.g., produced by and separated from) a parent cell. In some aspects, the BV is produced by a mammalian cell. The cell may be, e.g., an EXPI293F™ cell. In some aspects, the BV is an extracellular vesicle (EV), an exosome, a microvesicle, or a virus-like particle (VLP). In some aspects, BV preparations or compositions include mixtures of EVs, exosomes, microvesicles, and/or VLPs. BVs may be separated from parent cells and/or large EVs and protein aggregates (e.g., separated from the culture media of parent cells), e.g., using centrifugation (e.g., ultracentrifugation).

[0276] In some aspects, the parent cell has been transfected with a plasmid encoding the heterologous membrane-associated protein and a plasmid encoding the membrane-budding agent. The heterologous membrane-associated protein and the membrane-budding agent may be encoded by a single plasmid, or may be encoded by separate plasmids.

[0277] The level of expression of the heterologous membrane-associated protein and/or the membrane-budding agent on the surface of the parent cell and/or on the surface of the BV may be assessed. In some aspects, the level of expression of the heterologous membrane-associated protein is assessed using biolayer interferometry (BLI), wherein the BV produces at least a shift that is at or above a threshold level when contacted with an antibody against the tag associated with the heterologous membrane-associated protein. In some aspects, the tag is a gD polypeptide, the antibody is an anti-gD antibody, and the shift is at least 1.5 nm when the BLI assay is performed at 30° C. In other aspects, the BV is contacted with an antibody specific for the heterologous membrane-associated protein.

[0278] In some aspects, the BV comprises a marker that allows direct or indirect visualization of the BV, e.g., a membrane marker (e.g., a fluorescent membrane marker). In some aspects, the membrane marker is a cholesterol marker, e.g., AMPLEX™ Red.

III. Methods of Identifying Protein-Protein Interactions

[0279] Biological vesicles (BVs) provide a protein-purification free method for obtaining binding-competent receptors. The BVs bearing receptors may then be tested for interaction with ligands of the receptor (e.g., libraries of ligands), thus providing a method for identifying and assessing protein-protein interactions.

[0280] In some aspects, the disclosure features a method for identifying a protein-protein interaction, the method comprising (a) providing a collection of target polypeptides, optionally wherein the collection of target polypeptides is immobilized on one or more solid surfaces; (b) contacting the collection of step (a) with a biological vesicle (BV) comprising a heterologous membrane-associated protein and a membrane-budding agent under conditions permitting the binding of the heterologous membrane-associated protein and at least one of the target polypeptides, wherein the membrane budding agent is an HIV gag protein and wherein the heterologous membrane-associated protein is expressed at or above a threshold level on the surface of the BV; and (c) detecting an interaction between the heterologous membrane-associated protein and the at least one target polypeptide, thereby identifying a protein-protein interaction.

[0281] A. Heterologous Membrane-Associated Proteins

[0282] The heterologous membrane-associated protein may be any protein or polypeptide or fragment thereof that can be incorporated into an EV.

[0283] In some aspects, the heterologous membrane-associated protein is a full-length protein. In other aspects, the heterologous membrane-associated protein comprises a protein fragment, a tag, and an anchor. The protein fragment may be, e.g., an extracellular domain (e.g., an extracellular domain of a protein of interest, e.g., a transmembrane receptor. An ECD is a domain of a protein that is predicted to be localized outside of the plasma membrane of the cell. This domain of the protein is thus available to interact with the extracellular environment, e.g., interact with soluble proteins and ECDs of other proteins on the cell or on an adjacent cell. The ECD or ECDs of a protein may be identified by bioinformatics analysis, e.g., by analysis of UniProt annotations. For example, the boundaries of the ECD may be identified relative to the boundary of an adjacent predicted transmembrane region, e.g., a transmembrane helix. In some aspects, the presence of an extracellular domain may be predicted by the presence of a domain, sequence, or motif that indicates that the protein is trafficked to the plasma membrane, e.g., a signal sequence or a glycosylphosphatidylinositol (GPI) linkage site. In some aspects, the extracellular domain is expressed in the context of a full-length protein. In other aspects, the extracellular domain is expressed as an isolated extracellular domain, e.g., a sequence of amino acid residues comprising only the amino acid residues of a protein that are predicted to be extracellular. In some aspects, the isolated extracellular domain is expressed in a fusion protein.

[0284] In some aspects in which the heterologous membrane-associated protein comprises a protein fragment, a tag, and an anchor, the anchor tethers the protein fragment to the surface of a lipid membrane of a BV. In some aspects, the anchor is a glycosylphosphatidyl-inositol (GPI) polypeptide. In some aspects, the anchor is a moiety used in protein lipidation, e.g., a moiety used in cysteine palmitoylation, glycine myristoylation, lysine fatty-acylation, cholesterol esterification, cysteine prenylation, or serine fatty-acylation.

[0285] In some aspects in which the heterologous membrane-associated protein comprises a protein fragment, a tag, and an anchor, the tag can be directly or indirectly visualized, or otherwise detected. For example, the tag may comprise a moiety that can be detected using an antibody or an antibody fragment, e.g., may be a glycoprotein D (gD) polypeptide. In some aspects, the tag comprises a fluorescent protein.

[0286] In some aspects, the protein fragment is conjugated (e.g., fused) to a gD-GPI construct comprising a gD tag and a GPI anchor. In some aspects, the heterologous membrane-associated protein is a protein provided in Table 1 (e.g., an ECD of a protein provided in Table 1 conjugated to a gD-GPI construct) or a protein provided in Table 5, below.

TABLE 1

BV-expressed proteins
LRRC15
PD-L1/CD274
PVR
CD80/B7-1
CD276/B7-H3

[0287] B. BVs and Parent Cells

[0288] Biological vesicles (BVs) include any suitable lipid vesicle structure that has been derived from (e.g., produced by and separated from) a parent cell, as described in Section II(E) herein.

[0289] In some aspects, the parent cell has been transfected with a plasmid encoding the heterologous membrane-associated protein and a plasmid encoding the membrane-budding agent. The heterologous membrane-associated protein and the membrane-budding agent may be encoded by a single plasmid, or may be encoded by separate plasmids.

[0290] The level of expression of the heterologous membrane-associated protein and/or the membrane-budding agent on the surface of the parent cell and/or on the surface of the BV may be assessed. In some aspects, the level of expression of the heterologous membrane-associated protein is assessed using biolayer interferometry (BLI), wherein the BV produces at least a shift that is at or above a threshold

level when contacted with an antibody against the tag associated with the heterologous membrane-associated protein. In some aspects, the tag is a gD polypeptide, the antibody is an anti-gD antibody, and the threshold level is a shift of at least 1.5 nm when the BLI assay is performed at 30° C. In other aspects, the BV is contacted with an antibody specific for the heterologous membrane-associated protein.

[0291] In some aspects, the BV comprises a marker that allows direct or indirect visualization of the BV, e.g., a membrane marker (e.g., a fluorescent membrane marker). In some aspects, the membrane marker is a cholesterol marker, e.g., AMPLEX™ Red.

[0292] C. Membrane-Budding Agents

[0293] BVs may further comprise a membrane-budding agent that increases the production of BVs (e.g., extracellular vesicles (EVs), exosomes, microvesicles, and/or virus-like particles (VLPs)) by the parent cell, as described in Section II(D) herein.

[0294] In some aspects, the membrane-budding agent is an HIV gag protein. In some aspects, the HIV gag protein has the amino acid sequence of SEQ ID NO: 1. In some aspects, the HIV gag protein has at least 90% identity to the amino acid sequence of SEQ ID NO: 1, e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 1.

[0295] Further exemplary membrane budding agents include self-assembling VLPs (e.g., MLGag, AARDC1 (e.g., hAARDC1), and Acyl.Hrs); agents that enhance endogenous vesicle formation pathways such as exosome or tumor pathways (e.g., RhoA.F30L, ARF6.Q67L, VPS4a, HAS3, CD9, CD63, and CD81); and factors associated with apoptotic bodies (e.g., constitutively active ROCK1).

[0296] In some embodiments, the membrane budding agent is MLGag, Acyl.Hrs, ARRDC1 (e.g., hAARDC1), ARF6 (e.g., ARF6Q67L), RhoA (e.g., RhoA.F30L), or a combination thereof.

[0297] In some aspects, the membrane budding agent is a Gag protein, e.g., a chimeric Gag protein (e.g., a chimeric Gag protein as discussed in Hammarstedt et al., *J Virol.* 78(11): 5686-97, 2004 or Chen et al., *Proc Natl Acad Sci USA,* 98(26): 15239-44, 2001). In some aspects, the chimeric Gag protein comprises a portion of HIV Gag and a portion of Gag from a different retrovirus. For example, but not by way of limitation, the chimeric Gag comprises an HIV Gag, wherein a region of the HIV Gag known to direct its localization is replaced with functionally homologous regions from Moloney murine leukemia virus (MLV), a murine retrovirus. In certain embodiments, the replaced region of the HIV Gag is a matrix domain (MA), thus generating a chimeric Gag referred to herein as MLGag. In certain embodiments, chimeric and full-length Gag proteins can be generated from endogenous retrovirus (ERV) sequences derived from any species, e.g., as described in Stocking et al., *Cell Mol. Life Sci.,* 65(21):3383-3398, 2008. In certain embodiments, the vesicle factor is MLGag.

[0298] In certain embodiments, the vesicle factor is an arrestin domain-containing protein 1 (ARRDC1). In certain embodiments, the vesicle factor is a murine ARRDC1 (mARRDC1). In certain embodiments, the vesicle factor is a human ARRDC1 (hARRDC1). ARRDC1 is a tetrapeptide PSAP motif of an accessory protein and is a host protein that induces EV formation. It has been shown that overexpression of ARRDC 1 results in enhanced microvesicle (MV) formation. Such effect is mediated by the recruitment of Tsg 101 via PSAP/PTAP peptide. Overexpression of ATPase VP S4a results in further enhancement in MV formation (Nabhan et al., *Proc Natl Acad Sci USA,* 109(11): 4146-51, 2012).

[0299] In certain embodiments, the vesicle factor is ADP ribosylation factor-6 (ARF6). It has been shown that ARF6 is a Rho GTPase that drives microvesicle formation in tumor cells in an ERK-dependent manner (Muralidharan-Chari et al., *Curr Biol.,* 19(22): 1875-85, 2009. In certain embodiments, the vesicle factor is a constitutively active form of ARF6. For example, but not by way of limitation, the constitutively active form of ARF6 is ARF6.Q67L (see, e.g., Peters et al., *J. Cell Biol,* 128(6):1003-1017, 1995).

[0300] In certain embodiments, the vesicle factor is a mutant RhoA/ROCK1 that can also drive microvesicle formation in tumor cells (Li et al., *Oncogene,* 31(45): 4740-9, 2012). In certain embodiments, the vesicle factor is a constitutively active form of RhoA. For example, but not by way of limitation, the constitutively active form of RhoA is RhoA.F30L (see, e.g., Lin et al., *JBC,* 274(33): 23633-23641, 1999).

[0301] In certain embodiments, the vesicle factor comprises a plasma membrane (PM) binding domain, a self-assembly domain, and an endosomal sorting complex required for transport (ESCRT) recruiting domain. The design principle for EV formation is to enable rapid generation of new EV factors/cargo. It has been shown that PM targeting and high order oligomerization drives EV incorporation (Fang et al., *PLoS Biol.,* 5(6): e158, 2007. In certain embodiments, the vesicle factor is Acyl.Hrs that comprises a PM binding domain of acylation tag and the C-terminal domain of hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) that consists of a self-assembly domain of coiled coils, and an ESCRT recruiting domain. In certain embodiments, the vesicle factor is MLGag that comprises a PM binding domain of Matrix, a self-assembly domain of capsid, and an ESCRT recruiting domain of p6. In certain embodiments, the vesicle factor comprises a self-assembly domain and an ESCRT recruiting domain. In certain embodiments, the vesicle factor is ARRDC1 that comprises a self-assembly domain of arrestin domain, and an ESCRT recruiting domain.

[0302] Additional vesicle factors can be identified by any method known in the art. For example, but not by way of limitation, a screen of a cDNA library of all proteins, e.g., human proteins, can be performed to identify a single gene or a combination of genes that increases production of EVs. Alternatively or additionally, a CRISPR or RNAi screen can be performed to identify a single gene or a combination of genes that inhibits production of EVs.

[0303] In some aspects, the membrane-budding agent (e.g., HIV gag protein) comprises a marker that can be directly or indirectly visualized, or otherwise detected. In some aspects, the detectable marker is an enzyme that produces a fluorescent signal in the presence of a substrate, e.g., the enzyme is *Renilla* luciferase (Rluc) and the substrate is Rluc substrate.

[0304] Cells may additionally or alternatively be exposed to a condition (e.g., a culture condition) that increases the production of BVs (e.g., extracellular vesicles (EVs), exosomes, microvesicles, and/or virus-like particles (VLPs)) by the parent cell.

[0305] The membrane-budding agent and/or agent or condition that increases BV production may increase BV production by the parent cell by, e.g., 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, or more than 4-fold (e.g., 1.5 to

2.5-fold, 2.5 to 3.5-fold, or 3.5 to 4.5-fold). In some aspects, the membrane-budding agent (e.g., HIV gag protein) increases BV production by the parent cell by about 4-fold.

[0306] D. Collections of Target Polypeptides

[0307] In some aspects, the collection of target polypeptides is a collection of transmembrane receptors or fragments thereof. In some aspects, the receptors are single-pass transmembrane (STM) receptors. STM receptor proteins are a large category of membrane-bound receptors having a single domain passing through the plasma membrane. Many STM receptors are expressed on the cell surface, and thus may participate in the extracellular interactome. Exemplary STM receptors are provided in Tables 2 and 4 and in Martinez-Martin et al., *Cell,* 174(5): 1158-1171, 2018 and Clark et al., *Genome Res,* 13: 2265-2270, 2003.

[0308] In some aspects, the protein fragment is an extracellular domain (ECD), e.g., an ECD identified as described above. In some aspects, each member of the collection of target polypeptides is an Fc-tagged extracellular domain, and the solid surface is coated with protein A. In some aspects, one or more of the target polypeptides is immobilized to a distinct location on the one or more solid surfaces. In other aspects, the one or more target polypeptides are not immobilized to a surface.

TABLE 2

STM library proteins
TEM1/CD248/endosialin
PLXDC2
PTPRD
SARAF
ASGR1
BMP10
CPM
LDLR
PILRA
PRRG2
C6orf72
LRTM1
CDHR2
IGF2R
NCR3
SUSD3
CLEC17A
PVRL4
BTNL3
CDHR2
GLT8D2
KIAA1467
RNF152
LRFN1
MXRA5
PVRL1
LRIT2
PLA2R1
SLITRK4

TABLE 3

Interactions between BV-expressed proteins and STM library proteins	
Partner A	Partner B
LRRC15	TEM1/CD248/endosialin
	PLXDC2
	PTPRD
	SARAF

TABLE 3-continued

Interactions between BV-expressed proteins and STM library proteins	
Partner A	Partner B
	ASGR1
	BMP10
	CPM
	LDLR
	PILRA
	PRRG2
PD-L1/CD274	C6orf72
	LRTM1
	CDHR2
	IGF2R
	NCR3
	SUSD3
PVR	CLEC17A
	PVRL4
CD80/B7-1	BTNL3
	CDHR2
	GLT8D2
	KIAA1467
	RNF152
CD276/B7-H3	LRFN1
	MXRA5
	PVRL1
	LRIT2
	PLA2R1
	SLITRK4

TABLE 4

Tagged STM library	
Prey short name	Prey Entrez ID
1110032F04RIK	68725
A1BG	1
ACE	1636
ACE2	59272
ACPP	55
ACPT	93650
ACVR1	90
ACVR1B	91
ACVR1C	130399
ACVR2A	92
ACVR2B	93
ACVRL1	94
ADAM10	102
ADAM11	4185
ADAM12	8038
ADAM15	8751
ADAM17	6868
ADAM18	8749
ADAM19	8728
ADAM2	2515
ADAM20	8748
ADAM21	8747
ADAM22	53616
ADAM23	8745
ADAM28	10863
ADAM29	11086
ADAM30	11085
ADAM32	203102
ADAM33	80332
ADAM7	8756
ADAM8	101
ADAM9	8754
AGER	177
AJAP1	55966
ALCAM	214
ALK	238
ALPI	248

TABLE 4-continued

Tagged STM library

Prey short name	Prey Entrez ID
ALPL	249
ALPP	250
AMHR2	269
AMICA1	120425
AMIGO1	57463
AMIGO2	347902
AMIGO3	386724
AMN	81693
ANTXR1	84168
ANTXR2	118429
APCDD1	147495
APCDD1L	164284
APLP1	333
APLP2	334
APOO	79135
APP	351
AREG	374
ART1	417
ART3	419
ART4	420
ATRAID	51374
ATRN	8455
ATRNL1	26033
AXL	558
AZGP1	563
BACE1	23621
BACE2	25825
BAMBI	25805
BCAM	4059
BCAN	63827
BMPR1A	657
BMPR1B	658
BMPR2	659
BOC	91653
BSG	682
BST1	683
BST2	684
BTC	685
BTLA	151888
BTN1A1	696
BTN2A1	11120
BTN2A2	10385
BTN2A3P	54718
BTN3A1	11119
BTN3A2	11118
BTN3A3	10384
BTNL2	56244
BTNL3	10917
BTNL8	79908
BTNL9	153579
BUTR1	100129094
C10orf26	54838
C10orf35	219738
C10orf54	64115
C11orf24	53838
C11orf87	399947
C11orf92	399948
C12orf53	196500
C12orf59	120939
C14orf132	100132684
C14orf180	400258
C14orf37	145407
C15orf24	56851
C16orf54	728070
C16orf91	283951
C16orf92	146378
C17orf80	55028
C18orf1	753
C19orf18	147685
C19orf24	55009
C19orf38	255809
C19orf63	284361
C19orf75	284369

TABLE 4-continued

Tagged STM library

Prey short name	Prey Entrez ID
C1orf101	257044
C1orf130	400746
C1orf159	54991
C1orf85	112770
C2orf82	389084
C2orf89	129293
C3orf18	51161
C3orf35	339883
C3orf45	132228
C4orf32	132720
C4orf34	201895
C5orf15	56951
C6orf25	80739
C6orf72	116254
C9orf11	54586
CA12	771
CA14	23632
CA4	762
CA9	768
CACHD1	57685
CACNA2D3	55799
CACNA2D4	93589
CADM1	23705
CADM2	253559
CADM3	57863
CADM4	199731
CATSPERD	257062
CATSPERG	57828
CAV3	859
CCDC107	203260
CCDC47	57003
CD101	9398
CD109	135228
CD14	929
CD160	11126
CD163	9332
CD164	8763
CD164L2	388611
CD177	57126
CD180	4064
CD19	930
CD1A	909
CD1B	910
CD1C	911
CD1D	912
CD1E	913
CD2	914
CD200	4345
CD200R1	131450
CD200R1L	344807
CD22	933
CD226	10666
CD24	100133941
CD244	51744
CD247	919
CD248	57124
CD27	939
CD274	29126
CD276	80381
CD28	940
CD300A	11314
CD300C	10871
CD300E	342510
CD300LB	124599
CD300LD	100131439
CD300LF	146722
CD300LG	146894
CD302	9936
CD320	51293
CD33	945
CD34	947
CD3D	915
CD3E	916

TABLE 4-continued

Tagged STM library

Prey short name	Prey Entrez ID
CD3G	917
CD4	920
CD40	958
CD44	960
CD46	4179
CD47	961
CD48	962
CD5	921
CD52	1043
CD55	1604
CD58	965
CD6	923
CD68	968
CD7	924
CD79A	973
CD79B	974
CD80	941
CD83	9308
CD84	8832
CD86	942
CD8A	925
CD8B	926
CD93	22918
CD96	10225
CD99	4267
CD99L2	83692
CDCP1	64866
CDH1	999
CDH10	1008
CDH11	1009
CDH12	1010
CDH13	1012
CDH15	1013
CDH16	1014
CDH17	1015
CDH18	1016
CDH19	28513
CDH2	1000
CDH20	28316
CDH22	64405
CDH24	64403
CDH26	60437
CDH3	1001
CDH4	1002
CDH5	1003
CDH6	1004
CDH7	1005
CDH8	1006
CDH9	1007
CDHR3	222256
CDHR5	53841
CDON	50937
CEACAM1	634
CEACAM16	388551
CEACAM18	729767
CEACAM19	56971
CEACAM20	125931
CEACAM21	90273
CEACAM3	1084
CEACAM4	1089
CEACAM5	1048
CEACAM6	4680
CEACAM7	1087
CEACAM8	1088
CHL1	10752
CHODL	140578
CILP	8483
CILP2	148113
CLCA2	9635
CLCA4	22802
CLEC14A	161198
CLMP	79827
CLSTN1	22883

TABLE 4-continued

Tagged STM library

Prey short name	Prey Entrez ID
CLSTN2	64084
CLSTN3	9746
CNTFR	1271
CNTN1	1272
CNTN2	6900
CNTN3	5067
CNTN4	152330
CNTN5	53942
CNTN6	27255
CNTNAP1	8506
CNTNAP2	26047
CNTNAP3	79937
CNTNAP4	85445
CNTNAP5	129684
CPM	1368
CR1	1378
CR2	1380
CRB1	23418
CRB2	286204
CRB3	92359
CRIM1	51232
CRLF1	9244
CRLF2	64109
CRTAM	56253
CSF1	1435
CSF1R	1436
CSF2RA	1438
CSF2RB	1439
CSF3R	1441
CSPG4	1464
CSPG5	10675
CTLA4	1493
CUZD1	50624
CX3CL1	6376
CXADR	1525
CXCL16	58191
CXorf68	100132963
CYYR1	116159
DAG1	1605
DCBLD1	285761
DCBLD2	131566
DCC	1630
DDOST	1650
DDR1	780
DDR2	4921
DGCR2	9993
DLK1	8788
DLK2	65989
DLL1	28514
DLL3	10683
DLL4	54567
DNER	92737
DPCR1	135656
DPEP1	1800
DPEP2	64174
DPEP3	64180
DSC1	1823
DSC2	1824
DSC3	1825
DSCAM	1826
DSCAML1	57453
DSG1	1828
DSG2	1829
DSG3	1830
DSG4	147409
DTPQ5903	147645
ECSM2	641700
EDA2R	60401
EDAR	10913
EFNA3	1944
EFNA5	1946
EFNB1	1947
EFNB2	1948

TABLE 4-continued

Tagged STM library

Prey short name	Prey Entrez ID
EFNB3	1949
EGF	1950
EGFR	1956
ELFN1	392617
ELFN2	114794
EMB	133418
EMCN	51705
ENG	2022
ENPP5	59084
EPCAM	4072
EPGN	255324
EPHA1	2041
EPHA10	284656
EPHA2	1969
EPHA3	2042
EPHA4	2043
EPHA5	2044
EPHA6	285220
EPHA7	2045
EPHA8	2046
EPHB1	2047
EPHB2	2048
EPHB3	2049
EPHB4	2050
EPHB6	2051
EPOR	2057
ERBB2	2064
ERBB3	2065
ERBB4	2066
EREG	2069
ERMAP	114625
ERN2	10595
ESAM	90952
EVA1C	59271
EVC2	132884
EVI2A	2123
EVI2B	2124
F11R	50848
F3	2152
FAIM3	9214
FAM171A1	221061
FAM171B	165215
FAM174A	345757
FAM174B	400451
FAM187A	66784
FAM187B	148109
FAM189A2	9413
FAM200A	221786
FAM209A	200232
FAM209B	388799
FAS	355
FCAMR	83953
FCAR	2204
FCER1A	2205
FCGR1A	2209
FCGR1B	2210
FCGR1C	100132417
FCGR2A	2212
FCGR2B	2213
FCGR2C	9103
FCGR3A	2214
FCGR3B	2215
FCGRT	2217
FCRL1	115350
FCRL2	79368
FCRL3	115352
FCRL4	83417
FCRL5	83416
FCRL6	343413
FCRLA	84824
FCRLB	127943
FGFR1	2260
FGFR2	2263

TABLE 4-continued

Tagged STM library

Prey short name	Prey Entrez ID
FGFR3	2261
FGFR4	2264
FGFRL1	53834
FLRT1	23769
FLRT2	23768
FLRT3	23767
FLT1	2321
FLT3	2322
FLT3LG	2323
FLT4	2324
FNDC3A	22862
FNDC4	64838
FNDC9	408263
FOLR1	2348
FOLR2	2350
FRRS1L	23732
FSTL4	23105
FSTL5	56884
FURIN	5045
FXYD5	53827
GAS1	2619
GFRA1	2674
GFRA2	2675
GFRA3	2676
GFRA4	64096
GFRAL	389400
GHR	2690
GLG1	2734
GLIPR1	11010
GLIPR1L2	144321
GML	2765
GP1BA	2811
GP1BB	2812
GP2	2813
GP5	2814
GP6	51206
GP9	2815
GPA33	10223
GPC1	2817
GPC2	221914
GPC3	2719
GPC4	2239
GPC6	10082
GPIHBP1	338328
GPNMB	10457
GPR116	221395
GPR124	25960
GPR125	166647
GUCY2C	2984
GUCY2D	3000
GUCY2F	2986
GYPA	2993
GYPB	2994
GYPC	2995
GYPE	2996
HAPLN2	60484
HAPLN3	145864
HAPLN4	404037
HAVCR1	26762
HAVCR2	84868
HBEGF	1839
HCST	10870
HEG1	57493
HEPACAM	220296
HEPACAM2	253012
HEPH	9843
HEPHL1	341208
HFE	3077
HFE2	148738
HHLA2	11148
HYAL2	8692
ICAM1	3383
ICAM2	3384

TABLE 4-continued

Tagged STM library	
Prey short name	Prey Entrez ID
ICAM3	3385
ICAM4	3386
ICAM5	7087
ICOS	29851
ICOSLG	23308
IFNAR1	3454
IFNAR2	3455
IFNGR1	3459
IFNGR2	3460
IFNLR1	163702
IGDCC3	9543
IGDCC4	57722
IGF1R	3480
IGF2R	3482
IGFBP7	3490
IGFBPL1	347252
IGFLR1	79713
IGLON5	402665
IGSF1	3547
IGSF11	152404
IGSF21	84966
IGSF3	3321
IGSF5	150084
IGSF6	10261
IGSF8	93185
IGSF9	57549
IGSF9B	22997
IL10RA	3587
IL10RB	3588
IL11RA	3590
IL12B	3593
IL12RB1	3594
IL12RB2	3595
IL13RA1	3597
IL13RA2	3598
IL15RA	3601
IL17RA	23765
IL17RB	55540
IL17RC	84818
IL17RD	54756
IL17RE	132014
IL18BP	10068
IL18R1	8809
IL18RAP	8807
IL1R1	3554
IL1R2	7850
IL1RAP	3556
IL1RAPL1	11141
IL1RAPL2	26280
IL1RL1	9173
IL1RL2	8808
IL20RA	53832
IL20RB	53833
IL21R	50615
IL22RA1	58985
IL23R	149233
IL27RA	9466
IL2RA	3559
IL2RB	3560
IL2RG	3561
IL31RA	133396
IL3RA	3563
IL4R	3566
IL5RA	3568
IL6R	3570
IL6ST	3572
IL7R	3575
IL9R	3581
ILDR1	286676
ILDR2	387597
IMPG2	50939
INSR	3643
INSRR	3645

TABLE 4-continued

Tagged STM library	
Prey short name	Prey Entrez ID
ISLR	3671
ISLR2	57611
ITFG1	81533
ITGA1	3672
ITGA10	8515
ITGA11	22801
ITGA2	3673
ITGA2B	3674
ITGA3	3675
ITGA4	3676
ITGA5	3678
ITGA6	3655
ITGA7	3679
ITGA8	8516
ITGA9	3680
ITGAD	3681
ITGAE	3682
ITGAL	3683
ITGAM	3684
ITGAV	3685
ITGAX	3687
ITLN1	55600
IZUMO1	284359
IZUMO2	126123
JAG1	182
JAG2	3714
JAM2	58494
JAM3	83700
JTB	10899
KAZALD1	81621
KCNE4	23704
KDR	3791
KIAA0090	23065
KIAA0319	9856
KIAA0319L	79932
KIAA1024	23251
KIAA1324	57535
KIAA1324L	222223
KIAA1644	85352
KIR2DL1	3802
KIR2DL2	3803
KIR2DL3	3804
KIR2DL4	3805
KIR2DL5A	57292
KIR2DL5B	553128
KIR2DS1	3806
KIR2DS2	100132285
KIR2DS3	3808
KIR2DS4	3809
KIR2DS5	3810
KIR3DL1	3811
KIR3DL2	3812
KIR3DL3	115653
KIR3DP1	548594
KIR3DS1	3813
KIR3DX1	90011
KIRREL	55243
KIRREL2	84063
KIRREL3	84623
KIT	3815
KITLG	4254
KL	9365
KLB	152831
KLRAP1	10748
KREMEN1	83999
KREMEN2	79412
L1CAM	3897
LAG3	3902
LAIR1	3903
LAIR2	3904
LAMP1	3916
LAMP2	3920
LAMP3	27074

TABLE 4-continued

Tagged STM library	
Prey short name	Prey Entrez ID
LAMP5	24141
LAX1	54900
LAYN	143903
LCTL	197021
LDLR	3949
LDLRAD2	401944
LDLRAD3	143458
LEPR	3953
LIFR	3977
LILRA1	11024
LILRA2	11027
LILRA3	11026
LILRA4	23547
LILRA5	353514
LILRA6	79168
LILRB1	10859
LILRB2	10288
LILRB3	11025
LILRB4	11006
LILRB5	10990
LINGO1	84894
LINGO2	158038
LINGO3	645191
LINGO4	339398
LMLN	89782
LOC160348	160348
LOC256223	256223
LOC374383	374383
LOC376666	376666
LOC652900	652900
LRCH4	4034
LRFN1	57622
LRFN2	57497
LRFN3	79414
LRFN4	78999
LRFN5	145581
LRIG1	26018
LRIG2	9860
LRIG3	121227
LRIT1	26103
LRIT2	340745
LRIT3	345193
LRP10	26020
LRP11	84918
LRP12	29967
LRP3	4037
LRP4	4038
LRP5	4041
LRP6	4040
LRP8	7804
LRPAP1	4043
LRRC15	131578
LRRC19	64922
LRRC24	441381
LRRC25	126364
LRRC26	389816
LRRC3	81543
LRRC32	2615
LRRC33	375387
LRRC37A	9884
LRRC37A2	474170
LRRC37A3	374819
LRRC37B	114659
LRRC38	126755
LRRC3B	116135
LRRC4	64101
LRRC4B	94030
LRRC4C	57689
LRRC52	440699
LRRC55	219527
LRRC66	339977
LRRN1	57633
LRRN2	10446

TABLE 4-continued

Tagged STM library	
Prey short name	Prey Entrez ID
LRRN3	54674
LRRN4	164312
LRRN4CL	221091
LRRTM1	347730
LRRTM2	26045
LRRTM3	347731
LRRTM4	80059
LRTM1	57408
LRTM2	654429
LSAMP	4045
LSR	51599
LTBR	4055
LTK	4058
LY6D	8581
LY6E	4061
LY6G6C	80740
LY6G6D	58530
LY6G6F	259215
LY6H	4062
LY6K	54742
LY75	4065
LY9	4063
LYPD1	116372
LYPD3	27076
LYPD5	284348
LYPD6	130574
LYPD6B	130576
LYSMD3	116068
LYSMD4	145748
LYVE1	10894
M6PR	4074
MADCAM1	8174
MAG	4099
MAMDC4	158056
MANSC1	54682
MCAM	4162
MDGA1	266727
MDGA2	161357
MEGF10	84466
MEGF11	84465
MEGF8	1954
MEGF9	1955
MEP1A	4224
MEP1B	4225
MERTK	10461
MET	4233
MFAP3	4238
MFAP3L	9848
MFI2	4241
MICA	4276
MICB	4277
MILR1	28402
MMGT1	93380
MMP14	4323
MMP15	4324
MMP16	4325
MMP24	10893
MOG	4340
MPEG1	219972
MPL	4352
MPZ	4359
MPZL1	9019
MPZL2	10205
MPZL3	196264
MR1	3140
MRC1	4360
MRC2	9902
MSLN	10232
MST1R	4486
MUC1	4582
MUC13	56667
MUC15	143662
MUC21	394263

TABLE 4-continued

Tagged STM library	
Prey short name	Prey Entrez ID
MUSK	4593
MXRA5	25878
MXRA7	439921
MXRA8	54587
MYEOV	26579
NAGPA	51172
NCAM1	4684
NCAM2	4685
NCAN	1463
NCLN	56926
NCR1	9437
NCR2	9436
NCR3	259197
NCSTN	23385
NEGR1	257194
NEO1	4756
NETO1	81832
NETO2	81831
NFAM1	150372
NFASC	23114
NGFR	4804
NLGN1	22871
NLGN2	57555
NLGN3	54413
NLGN4X	57502
NLGN4Y	22829
NOMO1	23420
NOMO3	408050
NOTCH1	4851
NOTCH2	4853
NOTCH3	4854
NOTCH4	4855
NPDC1	56654
NPHS1	4868
NPR1	4881
NPR2	4882
NPR3	4883
NPTN	27020
NRCAM	4897
NRG1	3084
NRG2	9542
NRG4	145957
NRN1	51299
NRN1L	123904
NRP1	8829
NRP2	8828
NRXN1	9378
NRXN2	9379
NRXN3	9369
NT5E	4907
NTM	50863
NTNG1	22854
NTRK1	4914
NTRK2	4915
NTRK3	4916
OMG	4974
OPCML	4978
OSCAR	126014
OSMR	9180
OSTM1	28962
OTOA	146183
P2RX2	22953
PAPLN	89932
PARM1	25849
PCDHGB6	56100
PCDHGC3	5098
PCDHGC5	93708
PCSK4	54760
PCSK7	9159
PDCD1	5133
PDCD1LG2	80380
PDGFRA	5156
PDGFRB	5159

TABLE 4-continued

Tagged STM library	
Prey short name	Prey Entrez ID
PDGFRL	5157
PDPN	10630
PEAR1	375033
PECAM1	5175
Phospholipase Inhibitor	
PI16	221476
PIGR	5284
PIK3IP1	113791
PILRA	29992
PILRB	29990
PLA2R1	22925
PLB1	151056
PLXDC1	57125
PLXDC2	84898
PLXNA1	5361
PLXNA2	5362
PLXNA3	55558
PLXNA4	91584
PLXNB1	5364
PLXNB2	23654
PLXNB3	5365
PLXNC1	10154
PLXND1	23129
PMEL	6490
PMEPA1	56937
PODXL	5420
PODXL2	50512
PRIMA1	145270
PRLR	5618
PRND	23627
PRNP	5621
PROCR	10544
PRRG2	5639
PRRG3	79057
PRRG4	79056
PRSS21	10942
PRSS41	360226
PRSS55	203074
PRSS8	5652
PRTG	283659
PSG1	5669
PSG2	5670
PSG3	5671
PSG4	5672
PSG5	5673
PSG6	5675
PSG7	5676
PSG8	440533
PSG9	5678
PTCRA	171558
PTGFRN	5738
PTK7	5754
PTPRA	5786
PTPRB	5787
PTPRC	5788
PTPRCAP	5790
PTPRD	5789
PTPRE	5791
PTPRF	5792
PTPRG	5793
PTPRH	5794
PTPRJ	5795
PTPRK	5796
PTPRM	5797
PTPRN	5798
PTPRN2	5799
PTPRO	5800
PTPRR	5801
PTPRS	5802
PTPRT	11122
PTPRU	10076
PTPRZ1	5803

TABLE 4-continued

Tagged STM library

Prey short name	Prey Entrez ID
PVR	5817
PVRL1	5818
PVRL2	5819
NECTIN3	25945
PVRL4	81607
PXDN	7837
PXDNL	137902
QSOX1	5768
QSOX2	169714
RAET1E	135250
RAET1G	353091
RAET1L	154064
RAMP1	10267
RAMP2	10266
RAMP3	10268
RECK	8434
RELL1	768211
RELT	84957
RET	5979
RGMA	56963
RGMB	285704
ROBO1	6091
ROBO2	6092
ROBO3	64221
ROBO4	54538
ROR1	4919
ROR2	4920
ROS1	6098
RPN1	6184
RPRML	388394
RTN4R	65078
RTN4RL1	146760
RYK	6259
SARAF	51669
SCARF1	8578
SCARF2	91179
SCN1B	6324
SCN2B	6327
SCN3B	55800
SCN4B	6330
SDC1	6382
SDC2	6383
SDC3	9672
SDC4	6385
SDK1	221935
SDK2	237979
SECTM1	6398
SEL1L3	23231
SELE	6401
SELL	6402
SELP	6403
SELPLG	6404
SEMA3A	10371
SEMA3B	7869
SEMA3C	10512
SEMA3D	223117
SEMA3E	9723
SEMA3F	6405
SEMA3G	56920
SEMA4A	64218
SEMA4B	10509
SEMA4C	54910
SEMA4D	10507
SEMA4F	10505
SEMA4G	57715
SEMA5A	9037
SEMA5B	54437
SEMA6A	57556
SEMA6B	10501
SEMA6C	10500
SEMA6D	80031
SEMA7A	8482
SERTM1	400120

TABLE 4-continued

Tagged STM library

Prey short name	Prey Entrez ID
SEZ6	124925
SEZ6L	23544
SEZ6L2	26470
SGCA	6442
SGCE	8910
SHISA2	387914
SHISA3	152573
SHISA4	149345
SHISA5	51246
SIGIRR	59307
SIGLEC1	6614
SIGLEC10	89790
SIGLEC11	114132
SIGLEC12	89858
SIGLEC14	100049587
SIGLEC15	284266
SIGLEC16	400709
SIGLEC5	8778
SIGLEC6	946
SIGLEC7	27036
SIGLEC8	27181
SIGLEC9	27180
SIRPA	140885
SIRPB1	10326
SIRPB2	284759
SIRPD	128646
SIRPG	55423
SIT1	27240
SKINTL	391037
SLAMF1	6504
SLAMF6	114836
SLAMF7	57823
SLAMF8	56833
SLAMF9	89886
SLITRK1	114798
SLITRK2	84631
SLITRK3	22865
SLITRK4	139065
SLITRK5	26050
SLITRK6	84189
SMAGP	57228
SOGA3	387104
SORCS1	114815
SORCS2	57537
SORCS3	22986
SORL1	6653
SORT1	6272
SPACA1	81833
SPACA4	171169
SPATA9	83890
SPINT1	6692
SPINT2	10653
SPN	6693
SPRN	503542
STAB1	23166
STAB2	55576
STIM1	6786
SUSD1	64420
SUSD2	56241
SUSD3	203328
SUSD4	55061
SUSD5	26032
TACSTD2	4070
TAPBP	6892
TAPBPL	55080
TARM1	441864
TCP11	6954
TCTN2	79867
TCTN3	26123
TDGF1	6997
TECTA	7007
TECTB	6975
TEK	7010

TABLE 4-continued

Tagged STM library	
Prey short name	Prey Entrez ID
TEX101	83639
TEX29	121793
TGFA	7039
TGFBR1	7046
TGFBR2	7048
TGFBR3	7049
TGOLN2	10618
THBD	7056
THSD1	55901
THSD7A	221981
THSD7B	80731
THY1	7070
TIE1	7075
TIGIT	201633
TIMD4	91937
TLR1	7096
TLR10	81793
TLR2	7097
TLR3	7098
TLR4	7099
TLR5	7100
TLR6	10333
TLR7	51284
TLR8	51311
TLR9	54106
TMEFF1	8577
TMEFF2	23671
TMEM108	66000
TMEM119	338773
TMEM123	114908
TMEM130	222865
TMEM132A	54972
TMEM132B	114795
TMEM132D	121256
TMEM132E	124842
TMEM154	201799
TMEM156	80008
TMEM158	25907
TMEM167A	153339
TMEM167B	56900
TMEM183A	92703
TMEM183B	653659
TMEM190	147744
TMEM207	131920
TMEM213	155006
TMEM240	339453
TMEM25	84866
TMEM27	57393
TMEM52	339456
TMEM59	9528
TMEM59L	25789
TMEM81	388730
TMEM92	162461
TMEM95	339168
TMEM9B	56674
TMIE	259236
TMIGD1	388364
TMIGD2	126259
TMPRSS12	283471
TMX4	56255
TNFRSF10A	8797
TNFRSF10B	8795
TNFRSF10C	8794
TNFRSF10D	8793
TNFRSF11A	8792
TNFRSF11B	4982
TNFRSF12A	51330
TNFRSF13B	23495
TNFRSF13C	115650
TNFRSF14	8764
TNFRSF17	608
TNFRSF18	8784
TNFRSF19	55504

TABLE 4-continued

Tagged STM library	
Prey short name	Prey Entrez ID
TNFRSF1A	7132
TNFRSF1B	7133
TNFRSF21	27242
TNFRSF25	8718
TNFRSF4	7293
TNFRSF6B	8771
TNFRSF8	943
TNFRSF9	3604
TNFSF15	9966
TP53I13	90313
TPBG	7162
TPO	7173
TPSG1	25823
TREH	11181
TREM1	54210
TREM2	54209
TREML1	340205
TREML2	79865
TREML4	285852
TRIL	9865
TXNDC15	79770
TYRO3	7301
TYROBP	7305
ULBP1	80329
ULBP2	80328
ULBP3	79465
UMOD	7369
UMODL1	89766
UNC5A	90249
UNC5B	219699
UNC5C	8633
UNC5D	137970
UPK3BL	100134938
VASN	114990
VCAM1	7412
VLDLR	7436
VNN1	8876
VNN2	8875
VNN3	55350
VPREB1	7441
VPREB3	29802
VSIG1	340547
VSIG10	54621
VSIG2	23584
VSIG4	11326
VSTM1	284415
VSTM2A	222008
VSTM2B	342865
VSTM2L	128434
VSTM4	196740
VSTM5	387804
VTCN1	79679
WFIKKN1	117166
WFIKKN2	124857
XG	7499
XPNPEP2	7512
ZAN	7455
ZP1	22917
ZP2	7783
ZP3	7784
ZP4	57829
ZPBP	11055
ZPBP2	124626
ZPLD1	131368

[0309] In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 21%, at least 22%, at least 23%, at least 24%, at least 25%, at least 26%, at least 27%, at least 28%,

at least 29%, at least 30%, at least 31%, at least 32%, at least 33%, at least 34%, at least 35%, at least 36%, at least 37%, at least 38%, at least 39%, at least 40%, at least 41%, at least 42%, at least 43%, at least 44%, at least 45%, at least 46%, at least 47%, at least 48%, at least 49%, at least 50%, at least 51%, at least 52%, at least 53%, at least 54%, at least 55%, at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the proteins of Table 4, e.g., 5%-15%, 15%-25%, 25%-35%, 35%-455, 45%-55%, 55%-65%, 65%-75%, 75%-85%, 85%-95%, or 95%-100% of the proteins of Table 4.

[0310] In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, at least 600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, at least 1000, at least 1050, at least 1100, at least 1150, or all 1195 of the proteins of Table 4, e.g., comprises the extracellular domains of 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1050, 1050-1100, 1100-1150, or all 1195 of the polypeptides of Table 4.

[0311] In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 25% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 50% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 75% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 90% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of all of the proteins of Table 4.

[0312] In other aspects, the receptors are multi-transmembrane receptors (MTMRs), e.g., members of the GPCR superfamily.

[0313] E. Assays for Interaction

[0314] To perform the protein-protein interaction assay, the collection of target polypeptides (e.g., target polypeptides immobilized to a surface, e.g., target polypeptides immobilized in wells of a plate) is contacted with the BV comprising the heterologous membrane-associated protein (e.g., contacted with a solution comprising purified BVs). The assay may then be incubated and washed one or more times to remove non-bound BVs.

[0315] In some aspects, an interaction between the heterologous membrane-associated protein and the at least one target polypeptide is identified by detecting a signal at a location on the solid surface that is above a threshold level. The signal detected may be from one or more visualizable components of the BV, as follows.

[0316] In some aspects, the membrane-budding agent (e.g., HIV gag protein) further comprises (e.g., is conjugated to) a detectable marker, and detecting an interaction comprises detecting a level of the detectable marker at a location on the solid surface that is above a threshold level. In some aspects, the detectable marker is an enzyme that produces a fluorescent signal in the presence of a substrate. In some aspects, the enzyme is *Renilla* luciferase (Rluc), and the assay further comprises adding Rluc substrate, thus generating a fluorescent signal at a location on the solid surface at which an interaction has taken place.

[0317] In some aspects, the BV comprises a membrane marker, and detecting an interaction comprises detecting a level of the membrane marker at a location on the solid surface that is above a threshold level. In some aspects, the membrane marker is a cholesterol marker. In some aspects, the cholesterol marker is AMPLEX™ Red.

[0318] In some aspects, the interaction is a transient interaction.

[0319] In some aspects, the interaction is a low-affinity interaction.

[0320] In some aspects, the proteins provided in Table 1 and the STM proteins provided in Table 4 are tested for interaction in a protein-protein interaction assay as described above.

[0321] In some aspects, the assay described herein may identify the interactions provided in Table 3.

[0322] In some aspects, the assay described herein may identify interactions between LRRC15 and TEM1/CD248/endosialin, PLXDC2, PTPRD, SARAF, ASGR1, BMP10, CPM, LDLR, PILRA, and/or PRRG2.

[0323] In some aspects, the assay described herein may identify interactions between PD-L1/CD274 and C6orf72, LRTM1, CDHR2, IGF2R, NCR3, and/or SUSD3.

[0324] In some aspects, the assay described herein may identify interactions between PVR and CLEC17A and/or PVRL4.

[0325] In some aspects, the assay described herein may identify interactions between CD80/B7-1 and BTNL3, CDHR2, GLT8D2, KIAA1467, and/or RNF152.

[0326] In some aspects, the assay described herein may identify interactions between CD276/B7-H3 and LRFN1, MXRA5, PVRL1, LRIT2, PLA2R1, and/or SLITRK4. In some aspects, one or more multi-transmembrane receptors (MTMRs), e.g., members of the GPCR superfamily, are tested for interaction in a protein-protein interaction assay as described above.

[0327] In some aspects, the proteins provided in Table 5 and the proteins provided in Table 6 are tested for interaction in a protein-protein interaction assay as described above.

[0328] In some aspects, the assay described herein may identify the interactions provided in Table 7.

TABLE 5

Query proteins
ADGRB1
LGR4
LGR5

TABLE 6

Library proteins
PD-L1
ICOSLG

TABLE 6-continued

Library proteins
DNER
CNTN6
CLPS
EDIL3
IZUMO4
IZUMO1
BTNL3
CD93
CEACAM16
IL-6
LRRC4C
SCARF1
TRIL
CLPS
EDIL3
IZUMO4
CD93
GPR125
IL6R
SCARF1
TRIL

TABLE 7

Interactions between query proteins and library proteins

Partner A	Partner B
ADGRB1	PD-L1
	ICOSLG
	DNER
	CNTN6
LGR4	CLPS
	EDIL3
	IZUMO4
	IZUMO1
	BTNL3
	CD93
	CEACAM16
	IL-6
	LRRC4C
	SCARF1
	TRIL
LGR5	CLPS
	EDIL3
	IZUMO4
	CD93
	GPR125
	IL6R
	SCARF1
	TRIL

[0329] In some aspects, the assay described herein may identify interactions between ADGRB1 and PD-L1, ICOSLG, DNER, and/or CNTN6.

[0330] In some aspects, the assay described herein may identify interactions between LGR4 and CLPS, EDIL3, IZUMO4, IZUMO1, BTNL3, CD93, CEACAM16, IL-6, LRRC4C, SCARF1, and/or TRIL.

[0331] In some aspects, the assay described herein may identify interactions between LGR5 and CLPS, EDIL3, IZUMO4, CD93, GPR125, IL6-R, and/or TRIL.

[0332] F. BV-Protein Complexes

[0333] In another aspect, the disclosure features a protein complex comprising (a) a BV comprising a heterologous membrane-associated protein and a membrane-budding agent and (b) a target polypeptide, wherein the heterologous membrane-associated protein and the target polypeptide are bound to one another. Exemplary heterologous membrane-associated proteins and target polypeptides are described in Sections IIIA and IIID, respectively. In some aspects, the target polypeptide is immobilized on a surface and the complex is localized on the surface.

IV. Methods of Identifying a Modulator of a Protein-Protein Interaction

[0334] A. Assays for Modulation of Interaction

[0335] i. Proteins of Table 1 and Table 2

[0336] In some aspects, the disclosure features identifying a modulator of the interaction between a protein of Table 1 and a protein of Table 2, the method comprising: (a) providing a candidate modulator (e.g., a candidate modulator described in Section IV herein); (b) contacting a protein of Table 1 with a protein of Table 2 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 1 to the protein of Table 2, wherein the protein of Table 1 and the protein of Table 2 are reported to interact in Table 3; and (c) measuring the binding of the protein of Table 1 to the protein of Table 2, wherein an increase or decrease in binding in the presence of the candidate modulator relative to binding in the absence of the candidate modulator identifies the candidate modulator as a modulator of the interaction between the protein of Table 1 and the protein of Table 2.

[0337] ii. TEM1 and LRRC15

[0338] In some aspects, the disclosure features a method of identifying a modulator of the interaction between LRRC15 and TEM1, the method comprising (a) providing a candidate modulator; (b) contacting LRRC15 with TEM1 in the presence or absence of the candidate modulator under conditions permitting the binding of LRRC15 to TEM1; and (c) measuring the binding of LRRC15 to TEM1, wherein an increase or decrease in binding in the presence of the candidate modulator relative to binding in the absence of the candidate modulator identifies the candidate modulator as a modulator of the interaction between LRRC15 and TEM1.

[0339] iii. Proteins of Table 5 and Table 6

[0340] In some aspects, the disclosure features identifying a modulator of the interaction between a protein of Table 5 and a protein of Table 6, the method comprising: (a) providing a candidate modulator (e.g., a candidate modulator described in Section IV herein); (b) contacting a protein of Table 5 with a protein of Table 6 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 5 to the protein of Table 6, wherein the protein of Table 5 and the protein of Table 6 are reported to interact in Table 7; and (c) measuring the binding of the protein of Table 5 to the protein of Table 6, wherein an increase or decrease in binding in the presence of the candidate modulator relative to binding in the absence of the candidate modulator identifies the candidate modulator as a modulator of the interaction between the protein of Table 5 and the protein of Table 6.

[0341] iv. PD-L1 and ADGRB1

[0342] In some aspects, the disclosure features a method of identifying a modulator of the interaction between PD-L1 and ADGRB1, the method comprising (a) providing a candidate modulator; (b) contacting PD-L1 with ADGRB1 in the presence or absence of the candidate modulator under conditions permitting the binding of PD-L1 to ADGRB1; and (c) measuring the binding of PD-L1 to ADGRB1, wherein an increase or decrease in binding in the presence

of the candidate modulator relative to binding in the absence of the candidate modulator identifies the candidate modulator as a modulator of the interaction between PD-L1 and ADGRB1.

[0343] v. ICOSLG and ADGRB1

[0344] In some aspects, the disclosure features a method of identifying a modulator of the interaction between ICOSLG and ADGRB1, the method comprising (a) providing a candidate modulator; (b) contacting ICOSLG with ADGRB1 in the presence or absence of the candidate modulator under conditions permitting the binding of ICOSLG to ADGRB1; and (c) measuring the binding of ICOSLG to ADGRB1, wherein an increase or decrease in binding in the presence of the candidate modulator relative to binding in the absence of the candidate modulator identifies the candidate modulator as a modulator of the interaction between ICOSLG and ADGRB1.

[0345] vi. Assays for Modulation of Interaction

[0346] In some aspects, the candidate modulator is provided to a cell (e.g., a mammalian cell); to cell culture media; to conditioned media; to a purified form of a protein of Table 1 (e.g., a form of Protein 1 expressed on a BV) and/or a protein of Table 2; and/or to a purified form of a protein of Table 5 (e.g., a form of Protein 5 expressed on a BV) and/or a protein of Table 6. In some aspects, the candidate modulator is provided at a concentration of at least 0.1 nM, 0.5 nM, 1 nM, 10 nM, 50 nM, 100 nM, 250 nM, 500 nM, 750 nM, 1 μM, 2 μM, 3 μM, 5 μM, or 10 μM. In some aspects, the candidate modulator is provided at a concentration of between 0.1 nM and 10 μM. In some aspects, the candidate modulator is provided in a solution, e.g., in a soluble form.

[0347] In some aspects, the candidate modulator is identified as a modulator if the increase in binding is at least 70% (e.g., as measured by surface plasmon resonance, biolayer interferometry, or an enzyme-linked immunosorbent assay (ELISA). In some aspects, the increase in binding is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%). In some aspects, the increase in binding is at least 70%.

[0348] In some aspects, the candidate modulator is identified as a modulator if the decrease in binding is at least 70% (e.g., as measured by surface plasmon resonance, biolayer interferometry, or an enzyme-linked immunosorbent assay (ELISA)). In some aspects, the decrease in binding is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%). In some aspects, the decrease in binding is at least 70%. Exemplary methods for identifying modulators of protein-protein interactions, as well as agents that may modulate such interactions, are described below and in PCT/US2020/025471, which is hereby incorporated by reference in its entirety.

[0349] Modulation of the interaction between the protein of Table 1 and the protein of Table 2 or between the protein of Table 5 and the protein of Table 6 may be identified as an increase in protein-protein interaction in the presence of the modulator compared to protein-protein interaction in the absence of the modulator, e.g., an increase of 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 80%, 90%, 95%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%) in protein-protein interaction. Alternatively, modulation may be identified as a decrease in protein-protein interaction in the presence of the modulator compared to protein-protein interaction in the absence of the modulator, e.g., an decrease of 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 80%, 90%, 95%, or 100% (e.g., 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%) in protein-protein interaction. The assay for protein-protein interaction may be, e.g., a surface plasmon resonance (SPR) assay, a biolayer interferometry (BLI) assay, an enzyme-linked immunosorbent assay (ELISA), an extracellular interaction assay, or a cell surface interaction assay.

[0350] SPR Assays for Modulation of Protein-Protein Interaction

[0351] In some aspects, the assay for protein-protein interaction is a surface plasmon resonance (SPR) assay. In some aspects, modulation of the binding of the protein of Table 1 to the protein of Table 2 or of the protein of Table 5 to the protein of Table 6 is measured as a difference in SPR signal response units (RU) in the presence compared to the absence of the modulator.

[0352] BLI Assays for Modulation of Protein-Protein Interaction

[0353] In some aspects, the assay for protein-protein interaction is a biolayer interferometry (BLI) assay. In some aspects, the BLI assay is performed using isolated extracellular domains (ECDs). In some aspects, modulation of the binding of the protein of Table 1 to the protein of Table 2 or of the protein of Table 5 to the protein of Table 6 is measured as a difference in wavelength shift ($\Delta\lambda$) measured at a biosensor tip in the presence compared to the absence of the modulator.

[0354] ELISA for Modulation of Protein-Protein Interaction

[0355] In some aspects, the assay for protein-protein interaction is an enzyme-linked immunosorbent assay (ELISA). In some aspects, a first protein is bound to a plate (e.g., directly bound to a plate or bound to a plate via an affinity tag recognized by an antibody bound to a plate) and a second protein is provided in a soluble form, e.g., as an isolated ECD. An interaction between the first protein and the second protein may be detected by providing an antibody that binds to the second protein or to an affinity tag thereof, wherein the antibody can be detected, e.g., visualized, in an assay for presence of the antibody.

[0356] Other Assays for Modulation of Protein-Protein Interaction

[0357] In some aspects, the assay is an extracellular interaction assay, e.g., as described in PCT/US2020/025471, which is incorporated herein by reference in its entirety. In some aspects, the assay is a cell surface interaction assay, e.g., as described in PCT/US2020/025471. In some aspects, the assay is an isothermal titration calorimetry (ITC) assay, an assay comprising immunoprecipitation, or an assay comprising an ALPHASCREEN™ technology.

[0358] B. Assays for Changes in Downstream Activity

[0359] i. Proteins of Table 1 and Table 2

[0360] In some aspects, the disclosure features a method of identifying a modulator of a downstream activity of a protein of Table 1, the method comprising: (a) providing a candidate modulator (e.g., a candidate modulator described in Section IV herein); (b) contacting the protein of Table 1 with a protein of Table 2 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 1 to the protein of Table 2, wherein the protein of Table 1 and the protein of Table 2 are reported to interact in Table 3; and (c) measuring a downstream activity of the protein of Table 1, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of the protein of Table 1.

[0361] In some aspects, the disclosure features a method of identifying a modulator of a downstream activity of a protein of Table 2, the method comprising: (a) providing a candidate modulator (e.g., a candidate modulator described in Section IV herein); (b) contacting the protein of Table 2 with a protein of Table 1 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 2 to the protein of Table 1, wherein the protein of Table 1 and the protein of Table 2 are reported to interact in Table 3; and (c) measuring a downstream activity of the protein of Table 2, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of the protein of Table 2.

[0362] ii. TEM1 and LRRC15

[0363] In some aspects, the disclosure features a method of identifying a modulator of a downstream activity of LRRC15, the method comprising (a) providing a candidate modulator; (b) contacting LRRC15 with TEM1 in the presence or absence of the candidate modulator under conditions permitting the binding of LRRC15 to TEM1; and (c) measuring a downstream activity of LRRC15, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of LRRC15.

[0364] In some aspects, the disclosure features a method of identifying a modulator of a downstream activity of TEM1, the method comprising (a) providing a candidate modulator; (b) contacting TEM1 with LRRC15 in the presence or absence of the candidate modulator under conditions permitting the binding of TEM1 to LRRC15; and (c) measuring a downstream activity of TEM1, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of TEM1. In some aspects, the increase or decrease in binding is at least 70%, as measured by surface plasmon resonance, biolayer interferometry, or ELISA.

[0365] iii. Proteins of Table 5 and Table 6

[0366] In some aspects, the disclosure features a method of identifying a modulator of a downstream activity of a protein of Table 5, the method comprising: (a) providing a candidate modulator (e.g., a candidate modulator described in Section IV herein); (b) contacting the protein of Table 5 with a protein of Table 6 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 5 to the protein of Table 6, wherein the protein of Table 5 and the protein of Table 6 are reported to interact in Table 7; and (c) measuring a downstream activity of the protein of Table 5, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of the protein of Table 5.

[0367] In some aspects, the disclosure features a method of identifying a modulator of a downstream activity of a protein of Table 6, the method comprising: (a) providing a candidate modulator (e.g., a candidate modulator described in Section IV herein); (b) contacting the protein of Table 6 with a protein of Table 5 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 6 to the protein of Table 5, wherein the protein of Table 5 and the protein of Table 6 are reported to interact in Table 7; and (c) measuring a downstream activity of the protein of Table 6, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of the protein of Table 6.

[0368] iv. PD-L1 and ADGRB1

[0369] In some aspects, the disclosure features a method of identifying a modulator of a downstream activity of PD-L1, the method comprising (a) providing a candidate modulator; (b) contacting PD-L1 with ADGRB1 in the presence or absence of the candidate modulator under conditions permitting the binding of PD-L1 to ADGRB1; and (c) measuring a downstream activity of PD-L1, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of PD-1.

[0370] In some aspects, the disclosure features a method of identifying a modulator of a downstream activity of ADGRB1, the method comprising (a) providing a candidate modulator; (b) contacting ADGRB1 with PD-L1 in the presence or absence of the candidate modulator under conditions permitting the binding of ADGRB1 to PD-L1; and (c) measuring a downstream activity of ADGRB1, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of ADGRB1. In some aspects, the increase or decrease in binding is at least 70%, as measured by surface plasmon resonance, biolayer interferometry, or ELISA.

[0371] v. ICOSLG and ADGRB1

[0372] In some aspects, the disclosure features a method of identifying a modulator of a downstream activity of ICOSLG, the method comprising (a) providing a candidate modulator; (b) contacting ICOSLG with ADGRB1 in the presence or absence of the candidate modulator under conditions permitting the binding of ICOSLG to ADGRB1; and (c) measuring a downstream activity of ICOSLG, wherein a

change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of ICOSLG.

[0373] In some aspects, the disclosure features a method of identifying a modulator of a downstream activity of ADGRB1, the method comprising (a) providing a candidate modulator; (b) contacting ADGRB1 with ICOSLG in the presence or absence of the candidate modulator under conditions permitting the binding of ADGRB1 to ICOSLG; and (c) measuring a downstream activity of ADGRB1, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of ADGRB1. In some aspects, the increase or decrease in binding is at least 70%, as measured by surface plasmon resonance, biolayer interferometry, or ELISA.

[0374] vi. Assays for Changes in Downstream Activity

[0375] In some aspects, the candidate modulator is provided at a concentration of at least 0.1 nM, 0.5 nM, 1 nM, 10 nM, 50 nM, 100 nM, 250 nM, 500 nM, 750 nM, 1 μM, 2 μM, 3 μM, 5 μM, or 10 μM. In some aspects, the candidate modulator is provided at a concentration of between 0.1 nM and 10 μM. In some aspects, the candidate modulator is provided at a range of concentrations, e.g., as in FIG. **4**F. In some aspects, the candidate modulator is provided is provided in a solution, e.g., in a soluble form. In some aspects, the candidate modulator is provided to an organism comprising the protein of Table 1 and the protein of Table 2, to a tissue comprising the protein of Table 1 and the protein of Table 2, to a cell (e.g., a mammalian cell), to cell culture media, to conditioned media, and/or to a purified form of a protein of Table 1 and/or a protein of Table 2. In some aspects, the candidate modulator is provided to an organism comprising the protein of Table 5 and the protein of Table 6, to a tissue comprising the protein of Table 5 and the protein of Table 6, to a cell (e.g., a mammalian cell), to cell culture media, to conditioned media, and/or to a purified form of a protein of Table 5 and/or a protein of Table 6.

[0376] In some aspects, the modulator is an activator of the downstream activity of the protein of Table 1 or Table 2. In some aspects, the candidate modulator is identified as a modulator if the increase in the downstream activity of the protein of Table 1 or the protein of Table 2 is at least 30%. In some aspects, the modulator is an activator of the downstream activity of the protein of Table 5 or Table 6. In some aspects, the candidate modulator is identified as a modulator if the increase in the downstream activity of the protein of Table 5 or the protein of Table 6 is at least 30%. In some aspects, the increase in the downstream activity is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, or more than 100% (e.g., at least 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%). In some aspects, the increase in the downstream activity is at least 30%. In some aspects, the change in the downstream activity is an increase in the amount, strength, or duration of the downstream activity.

[0377] In some aspects, the modulator is an inhibitor of the downstream activity of the protein of Table 1 or Table 2. In some aspects, the candidate modulator is identified as a modulator if the decrease in the downstream activity of the protein of Table 1 or the protein of Table 2 is at least 30%. In some aspects, the modulator is an inhibitor of the downstream activity of the protein of Table 5 or Table 6. In some aspects, the candidate modulator is identified as a modulator if the decrease in the downstream activity of the protein of Table 5 or the protein of Table 6 is at least 30%. In some aspects, the decrease in the downstream activity is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% (e.g., at least 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%). In some aspects, the decrease in downstream activity is at least 30%. In some aspects, the change in the downstream activity is a decrease in the amount, strength, or duration of the downstream activity.

[0378] In some aspects, the downstream activity of the protein of Table 1 or the protein of Table 2 is assessed in one or more assays. In some aspects, the downstream activity of the protein of Table 5 or the protein of Table 6 is assessed in one or more assays.

[0379] In some aspects, the downstream activity is an activity relating to the development or progression of a disease, e.g., a cancer.

[0380] In some aspects, the downstream activity is tumor growth. In some aspects, the protein of Table 1 is LRRC15, the protein of Table 2 is TEM1, and the downstream activity is tumor growth. In some aspects, tumor growth is decreased by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% (e.g., at least 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%) in the presence of the modulator, as measured in a tumor growth assay. In some aspects, tumor growth is decreased by at least 20% in the presence of the modulator, as measured in a tumor growth assay.

[0381] In some aspects, the protein of Table 5 is ADGRB, the protein of Table 2 is PD-L1, and the downstream activity is tumor growth. In some aspects, tumor growth is decreased by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% (e.g., at least 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%) in the presence of the modulator, as measured in a tumor growth assay. In some aspects, tumor growth is decreased by at least 20% in the presence of the modulator, as measured in a tumor growth assay.

[0382] In some aspects, the protein of Table 5 is ADGRB, the protein of Table 2 is PD-L1, and the downstream activity is engulfment of bacterial cells or apoptotic cells. In some aspects, the rate of engulfment is increased by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% (e.g., at least 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%) in the presence of the modulator.

[0383] In some aspects, the protein of Table 5 is ADGRB, the protein of Table 2 is ICOSLG, and the downstream activity is T cell activation. In some aspects, T cell activation is increased by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% (e.g., at least 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%,

70%-80%, 80%-90%, or 90%-100%). In some aspects, T cell activation is increased by at least 20% in the presence of the modulator.

[0384] C. Small Molecules

[0385] In some aspects, the modulator or candidate modulator is a small molecule. Small molecules are molecules other than binding polypeptides or antibodies as defined herein that may bind, preferably specifically, to a protein of Table 1 and/or a protein of Table 2 or to a protein of Table 5 and/or a protein of Table 6. Binding small molecules may be identified and chemically synthesized using known methodology (see, e.g., PCT Publication Nos. WO00/00823 and WO00/39585). Binding small molecules are usually less than about 2000 daltons in size (e.g., less than about 2000, 1500, 750, 500, 250 or 200 daltons in size), wherein such organic small molecules that are capable of binding, preferably specifically, to a polypeptide as described herein may be identified without undue experimentation using well known techniques. In this regard, it is noted that techniques for screening small molecule libraries for molecules that are capable of binding to a polypeptide target are well known in the art (see, e.g., PCT Publication Nos. WO00/00823 and WO00/39585). Binding small molecules may be, for example, aldehydes, ketones, oximes, hydrazones, semicarbazones, carbazides, primary amines, secondary amines, tertiary amines, N-substituted hydrazines, hydrazides, alcohols, ethers, thiols, thioethers, disulfides, carboxylic acids, esters, amides, ureas, carbamates, carbonates, ketals, thioketals, acetals, thioacetals, aryl halides, aryl sulfonates, alkyl halides, alkyl sulfonates, aromatic compounds, heterocyclic compounds, anilines, alkenes, alkynes, diols, amino alcohols, oxazolidines, oxazolines, thiazolidines, thiazolines, enamines, sulfonamides, epoxides, aziridines, isocyanates, sulfonyl chlorides, diazo compounds, acid chlorides, or the like.

[0386] In some aspects, the binding of a protein of Table 1 and a protein of Table 2 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the small molecule. In some aspects, the binding of a protein of Table 1 and a protein of Table 2 is increased (e.g., increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the small molecule. In some aspects, a downstream activity of the protein of Table 1 and/or the protein of Table 2 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the small molecule. In some aspects, a downstream activity of the protein of Table 1 and/or the protein of Table 2 is increased (e.g., increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the small molecule.

[0387] In some aspects, the binding of a protein of Table 5 and a protein of Table 6 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the small molecule. In some aspects, the binding of a protein of Table 5 and a protein of Table 6 is increased (e.g., increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the small molecule. In some aspects, a downstream activity of the protein of Table 5 and/or the protein of Table 6 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the small molecule. In some aspects, a downstream activity of the protein of Table 5 and/or the protein of Table 6 is increased (e.g., increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the small molecule.

[0388] D. Antibodies and Antigen-Binding Fragments

[0389] In some aspects, the modulator or candidate modulator is an antibody or an antigen-binding fragment thereof binding a protein of Table 1 and/or a protein of Table 2. In some aspects, the antigen-binding fragment is a bis-Fab, an Fv, a Fab, a Fab'-SH, a $F(ab')_2$, a diabody, a linear antibody, an scFv, an ScFab, a VH domain, or a VHH domain. In some aspects, the antibody or antigen-binding fragment thereof binds the protein of Table 1. In other aspects, the antibody or antigen-binding fragment thereof binds the protein of Table 2.

[0390] In some aspects, the binding of a protein of Table 1 and a protein of Table 2 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the antibody or antigen-binding fragment. In some aspects, the binding of a protein of Table 1 and a protein of Table 2 is increased (e.g., increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the antibody or antigen-binding fragment. In some aspects, a downstream activity (e.g., a downstream activity described in Section IIIB herein, e.g., CAF contractility, immune checkpoint inhibition, suppression of cell proliferation, modulation of target phosphorylation, inhibition of cell migration, suppression of tumor formation, suppression of cell invasion, macrophage polarization, regulation of phagocytosis, osteoclast differentiation, activation of a signaling pathway, or formation of filopodia) of the protein of Table 1 and/or the protein of Table 2 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the antibody or antigen-binding fragment. In some aspects, a downstream activity (e.g., a downstream activity described in Section IIIB herein) of the protein of Table 1 and/or the protein of Table 2 is increased (e.g.,

increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the antibody or antigen-binding fragment.

[0391] In some aspects, the modulator or candidate modulator is an antibody or an antigen-binding fragment thereof binding a protein of Table 5 and/or a protein of Table 6. In some aspects, the antigen-binding fragment is a bis-Fab, an Fv, a Fab, a Fab'-SH, a $F(ab')_2$, a diabody, a linear antibody, an scFv, an ScFab, a VH domain, or a VHH domain. In some aspects, the antibody or antigen-binding fragment thereof binds the protein of Table 5. In other aspects, the antibody or antigen-binding fragment thereof binds the protein of Table 6.

[0392] In some aspects, the binding of a protein of Table 5 and a protein of Table 6 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the antibody or antigen-binding fragment. In some aspects, the binding of a protein of Table 5 and a protein of Table 6 is increased (e.g., increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the antibody or antigen-binding fragment. In some aspects, a downstream activity (e.g., a downstream activity described in Section IIIB herein, e.g., CAF contractility, immune checkpoint inhibition, suppression of cell proliferation, modulation of target phosphorylation, inhibition of cell migration, suppression of tumor formation, suppression of cell invasion, macrophage polarization, regulation of phagocytosis, osteoclast differentiation, activation of a signaling pathway, or formation of filopodia) of the protein of Table 5 and/or the protein of Table 6 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the antibody or antigen-binding fragment. In some aspects, a downstream activity (e.g., a downstream activity described in Section IIIB herein) of the protein of Table 5 and/or the protein of Table 6 is increased (e.g., increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the antibody or antigen-binding fragment.

[0393] E. Peptides

[0394] In some aspects, the modulator or candidate modulator is a peptide that binds to a protein of Table 1 and/or a protein of Table 2. The peptide may be the peptide may be naturally occurring or may be engineered. In some aspects, the peptide is a fragment of the protein of Table 1, the protein of Table 2, or another protein that binds to the protein of Table 1 or the protein of Table 2. The peptide may bind the binding partner with equal, less, or greater affinity than the full-length protein. In some aspects, the peptide performs all functions of the full-length protein. In other aspects, the peptide does not perform all functions of the full-length protein.

[0395] In some aspects, the binding of a protein of Table 1 and a protein of Table 2 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the peptide. In some aspects, the binding of a protein of Table 1 and a protein of Table 2 is increased (e.g., increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the peptide. In some aspects, a downstream activity of the protein of Table 1 and/or the protein of Table 2 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the peptide. In some aspects, a downstream activity of the protein of Table 1 and/or the protein of Table 2 is increased (e.g., increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the peptide.

[0396] In some aspects, the modulator or candidate modulator is a peptide that binds to a protein of Table 5 and/or a protein of Table 6. The peptide may be the peptide may be naturally occurring or may be engineered. In some aspects, the peptide is a fragment of the protein of Table 5, the protein of Table 6, or another protein that binds to the protein of Table 5 or the protein of Table 6. The peptide may bind the binding partner with equal, less, or greater affinity than the full-length protein. In some aspects, the peptide performs all functions of the full-length protein. In other aspects, the peptide does not perform all functions of the full-length protein.

[0397] In some aspects, the binding of a protein of Table 5 and a protein of Table 6 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the peptide. In some aspects, the binding of a protein of Table 5 and a protein of Table 6 is increased (e.g., increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the peptide. In some aspects, a downstream activity of the protein of Table 5 and/or the protein of Table 6 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the peptide. In some aspects, a downstream activity of the protein of Table 5 and/or the protein of Table 6 is increased (e.g., increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the peptide.

[0398] F. Mimics

[0399] In some aspects, the modulator or candidate modulator is a mimic, e.g., a molecular mimic, that binds to a protein of Table 1 and/or a protein of Table 2. The mimic may be a molecular mimic of the protein of Table 1, the protein of Table 2, or another protein that binds to the protein of Table 1 or the protein of Table 2. In some aspects, the mimic may perform all functions of the mimicked polypeptide. In other aspects, the mimic does not perform all functions of the mimicked polypeptide.

[0400] In some aspects, the binding of a protein of Table 1 and a protein of Table 2 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the mimic. In some aspects, the binding of a protein of Table 1 and a protein of Table 2 is increased (e.g., increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the mimic. In some aspects, a downstream activity of the protein of Table 1 and/or the protein of Table 2 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the mimic. In some aspects, a downstream activity of the protein of Table 1 and/or the protein of Table 2 is increased (e.g., increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the mimic.

[0401] In some aspects, the modulator or candidate modulator is a mimic, e.g., a molecular mimic, that binds to a protein of Table 5 and/or a protein of Table 6. The mimic may be a molecular mimic of the protein of Table 5, the protein of Table 6, or another protein that binds to the protein of Table 5 or the protein of Table 6. In some aspects, the mimic may perform all functions of the mimicked polypeptide. In other aspects, the mimic does not perform all functions of the mimicked polypeptide.

[0402] In some aspects, the binding of a protein of Table 5 and a protein of Table 6 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the mimic. In some aspects, the binding of a protein of Table 5 and a protein of Table 6 is increased (e.g., increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the mimic. In some aspects, a downstream activity of the protein of Table 5 and/or the protein of Table 6 is decreased (e.g., decreased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%)) in the presence of the mimic. In some aspects, a downstream activity of the protein of Table 5 and/or the protein of Table 6 is increased (e.g., increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% (e.g., 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, 80%-90%, 90%-100%, or more than 100%)) in the presence of the mimic.

V. Methods of Treatment Comprising Modulators of Identified Protein-Protein Interactions

[0403] In some aspects, a modulator of a protein-protein interaction described herein is used to treat or delay progression of a pathological state, disease, disorder, or condition, e.g., a cancer.

[0404] In some aspects, the modulator increases or decreases the amount, strength, or duration of a downstream activity of the protein-protein interaction, e.g., tumor formation or tumor growth, in an individual to whom the modulator has been administered.

[0405] A. Cancers

[0406] In some aspects, a modulator of a protein-protein interaction described herein (e.g., a modulator of the interaction between LRRC15 and TEM1; a modulator of an interaction between PD-L1 and ADGRB1; or a modulator of an interaction between ADGRB1 and ICOSLG), e.g., a small molecule, an antibody, an antigen-binding fragment, a peptide, a mimic, an antisense oligonucleotide, or an siRNA, is used to treat or delay progression of a cancer in a subject in need thereof. In some aspects, the subject is a human. The cancer may be a solid tumor cancer or a non-solid tumor cancer. Solid cancer tumors include, but are not limited to a bladder cancer, a melanoma, a breast cancer, a colorectal cancer, a lung cancer, a head and neck cancer, a kidney cancer, an ovarian cancer, a pancreatic cancer, or a prostate cancer, or metastatic forms thereof. In some aspects, the cancer is a bladder cancer. Further aspects of bladder cancer include urothelial carcinoma, muscle invasive bladder cancer (MIBC), or non-muscle invasive bladder cancer (NMIBC). In some aspects, the bladder cancer is a metastatic urothelial carcinoma (mUC). In some aspects, the cancer is a breast cancer. Further aspects of breast cancer include a hormone receptor-positive (HR+) breast cancer, e.g., an estrogen receptor-positive (ER+) breast cancer, a progesterone receptor-positive (PR+) breast cancer, or an ER+/PR+ breast cancer. Other aspects of breast cancer include a HER2-positive (HER2+) breast cancer. Yet other aspects of breast cancer include a triple-negative breast cancer (TNBC). In some aspects, the breast cancer is an early breast cancer. In some aspects, the cancer is a lung cancer. Further aspects of lung cancer include an epidermal growth factor receptor-positive (EGFR+) lung cancer. Other aspects of lung cancer include an epidermal growth factor receptor-negative (EGFR−) lung cancer. Yet other aspects of lung cancer include a non-small cell lung cancer, e.g., a squamous lung cancer or a non-squamous lung cancer. Other aspects of lung cancer include a small cell lung cancer. In some aspects, the cancer is a head and neck cancer. Further aspects of head and neck cancer include a squamous cell carcinoma of the head & neck (SCCHN). In some aspects, the cancer is a kidney cancer. Further aspects of kidney cancer include a renal cell carcinoma (RCC). In some aspects, the cancer is a liver cancer. Further aspects of liver cancer include a hepatocellular carcinoma. In some aspects, the cancer is a prostate cancer. Further aspects of prostate cancer include a castration-resistant prostate cancer (CRPC). In some aspects, the cancer is a metastatic form of a solid tumor. In some aspects, the metastatic form of a solid tumor is a metastatic form of a melanoma, a breast cancer, a

colorectal cancer, a lung cancer, a head and neck cancer, a bladder cancer, a kidney cancer, an ovarian cancer, a pancreatic cancer, or a prostate cancer. In some aspects, the cancer is a metastatic urothelial carcinoma (mUC). In some aspects, the cancer is a non-solid tumor cancer. Non-solid tumor cancers include, but are not limited to, a B-cell lymphoma. Further aspects of B-cell lymphoma include, e.g., a chronic lymphocytic leukemia (CLL), a diffuse large B-cell lymphoma (DLBCL), a follicular lymphoma, myelodysplastic syndrome (MDS), a non-Hodgkin lymphoma (NHL), an acute lymphoblastic leukemia (ALL), a multiple myeloma, an acute myeloid leukemia (AML), or a mycosis fungoides (MF).

[0407] B. Combination Therapies

[0408] In some aspects of the above-described methods of treatment and prophylaxis, the method comprises administering to the individual at least one additional therapy (e.g., one, two, three, four, or more than four additional therapies). The modulator may be administered to the individual prior to, concurrently with, or after the at least one additional therapy.

[0409] C. Methods of Delivery

[0410] The compositions utilized in the methods described herein (e.g., a modulator of a protein-protein interaction described herein, e.g., a small molecule, an antibody, an antigen-binding fragment, a peptide, a mimic, an antisense oligonucleotide, or an siRNA) can be administered by any suitable method, including, for example, intravenously, intramuscularly, subcutaneously, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intrathecally, intranasally, intravaginally, intrarectally, topically, intratumorally, peritoneally, subconjunctivally, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, intraorbitally, orally, transdermally, intravitreally (e.g., by intravitreal injection), by eye drop, by inhalation, by injection, by implantation, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, in cremes, or in lipid compositions. The compositions utilized in the methods described herein can also be administered systemically or locally. The method of administration can vary depending on various factors (e.g., the compound or composition being administered and the severity of the condition, disease, or disorder being treated). In some aspects, a modulator of a protein-protein interaction is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. Dosing can be by any suitable route, e.g., by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0411] A modulator of a protein-protein interaction described herein (and any additional therapeutic agent) may be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The modulator need not be, but is optionally formulated with and/or administered concurrently with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of the modulator present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

VI. Methods of Identifying Biological Vesicles Having Altered Binding Profiles

[0412] In some aspects, the disclosure features a method for identifying a biological vesicle (BV) having an altered binding profile, the method comprising (a) providing a collection of target polypeptides that are immobilized on one or more solid surfaces; (b) contacting the collection of step (a) with a BV of interest; (c) detecting an interaction between the BV of interest and the at least one target polypeptide, thereby identifying an interaction profile; and (d) comparing the interaction profile of the BV of interest to the interaction profile of a control BV, wherein a difference between the interaction profile of the BV of interest and the interaction profile of the control BV identifies the BV of interest as one having an altered binding profile.

[0413] In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 21%, at least 22%, at least 23%, at least 24%, at least 25%, at least 26%, at least 27%, at least 28%, at least 29%, at least 30%, at least 31%, at least 32%, at least 33%, at least 34%, at least 35%, at least 36%, at least 37%, at least 38%, at least 39%, at least 40%, at least 41%, at least 42%, at least 43%, at least 44%, at least 45%, at least 46%, at least 47%, at least 48%, at least 49%, at least 50%, at least 51%, at least 52%, at least 53%, at least 54%, at least 55%, at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the proteins of Table 4, e.g., 5%-15%, 15%-25%, 25%-35%, 35%-455, 45%-55%, 55%-65%, 65%-75%, 75%-85%, 85%-95%, or 95%-100% of the proteins of Table 4.

[0414] In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, at least 600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, at least 1000, at least 1050, at least 1100, at least 1150, or all 1195 of the proteins of Table 4, e.g., comprise the extracellular domains of 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700,

750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1050, 1050-1100, 1100-1150, or all 1195 of the polypeptides of Table 4.

[0415] In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 25% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 50% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 75% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 90% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of all of the proteins of Table 4.

[0416] In some aspects, the extracellular domain of the prey protein (e.g., STM protein) has a native conformation, e.g., a conformation observed in the wild-type protein. In some aspects, the extracellular domain of the prey protein (e.g., STM protein) comprises a native post-translational modification.

[0417] In some aspects, the BV of interest is an engineered BV, e.g., a BV derived from a parent cell that has been modified (e.g., modified to express a heterologous protein, e.g., a receptor). The control BV may be, e.g., a BV produced by a control process or a BV derived from an unmodified parent cell.

[0418] In some aspects, the BV is a BV for use as a drug delivery vehicle. The control BV may be, e.g., a BV having a desired characteristic of a drug delivery vehicle. In some aspects, the method is used for quality control, e.g., to detect unexpected changes in the binding profile of EVs (e.g., changes arising from the overexpression of target receptors, addition of therapeutics, or modifications of the cells generating the EVs).

[0419] In some aspects, the BV of interest is derived from a sample from a subject. In some aspects, the method is used for unbiased profiling of the receptors for EVs from samples from a subject (e.g., used for comparison of EVs produced by different tissues, cell types, tumor cell lines, or immune cells. In some aspects, the BV of interest and the control BV are derived from different tissues or different cell types. In some aspects, the BV of interest is derived from a diseased tissue (e.g., tumor tissue), and the control BV is derived from healthy tissue.

VII. Methods of Characterizing Interaction Profiles of Cell Lines

[0420] A. Methods of Characterizing Interaction Profiles of Cell Lines

[0421] In some aspects, the disclosure features a method for characterizing an interaction profile of a cell line, the method comprising (a) modifying the cell line to comprise a membrane-budding agent; and (b) characterizing an interaction profile of a biological vesicle (BV) produced by the cell line.

[0422] In some aspects, the disclosure features a method for characterizing an interaction profile of a cell line that has been modified to comprise a membrane-budding agent, the method comprising characterizing an interaction profile of a BV produced by the cell line.

[0423] In some aspects, the cell line may be a mammalian cell line. In some aspects, the mammalian cell line is a neuronal cell line, a fibroblast cell line, or an immune cell line. In some aspects, the immune cell line comprises one or more of T-cells, B-cells, or monocytes (e.g., consists of T-cell, or consists of B-cells, or consists of monocytes), e.g., is a T cell line, a B cell line, or a monocyte cell line.

[0424] In some aspects, the cell line is a mammalian cell line representing (e.g., derived from) a tissue type of interest, (e.g., a diseased or healthy tissue type) or a cell type of interest or a cell line representing (e.g., derived from) a tumor of interest (e.g., a tumor cell line). In some aspects, the cell line (e.g., mammalian cell line) is derived from a sample from a subject, e.g., a subject having a disease.

[0425] Exemplary membrane-budding agents are provided in Section III(C) herein. In some aspects, the membrane-budding agent is selected from the group consisting of a HIV gag protein, Acyl.Hrs, ARRDC1, and ARF6. In some aspects, the membrane-budding agent is a HIV gag protein. In some aspects, expression of the membrane-budding agent in the cell line is inducible. Exemplary constructs for inducible expression of the membrane-budding agent that may be introduced into the cell line include (a) an inducible promoter that relieves or suppresses the expression of the membrane-budding agent after the addition of a small molecule (e.g., a cell-permeable small molecule), e.g., the T-REX™ System; (b) a small molecule-induced degradation system in which the membrane-budding agent is rapidly degraded upon induction (e.g., the TIR1 auxin inducible degron (AID) system); and (c) a small molecule-induced stabilization system in which the membrane-budding agent comprises a degradation domain and the protein is protected from degradation upon induction (e.g., the Shield-1-FKBP system). Integration of constructs can be performed, e.g., by using CRISPR-Cas9 or genome engineering techniques to insert the construct into a safe-harbor locus. Alternatively, methods of random integration such as the PiggyBac Transposon System (SBI) may be used. Expression of the membrane-budding agent in the cell line may be induced at any desired time point; thus, production of BVs be the cell line may be induced at any time point.

[0426] In some aspects, characterizing the interaction profile of the BV comprises determining a level of one or more membrane-associated proteins of interest (e.g., one or more receptors of interest) on the BV.

[0427] In some aspects, characterizing the interaction profile of the BV is performed using a method comprising (a) providing a collection of target polypeptides that are immobilized on one or more solid surfaces; (b) contacting the collection of target polypeptides in step (a) with the BV; and (c) detecting an interaction between the BV and the at least one target polypeptide of the collection of target polypeptides, thereby identifying an interaction profile.

[0428] In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 21%, at least 22%, at least 23%, at least 24%, at least 25%, at least 26%, at least 27%, at least 28%, at least 29%, at least 30%, at least 31%, at least 32%, at least 33%, at least 34%, at least 35%, at least 36%, at least 37%, at least 38%, at least 39%, at least 40%, at least 41%, at least 42%, at least 43%, at least 44%, at least 45%, at least 46%, at least 47%, at least 48%, at least 49%, at least 50%, at least 51%, at least 52%, at least 53%, at least 54%, at least 55%, at least 56%, at least 57%, at least 58%, at least 59%, at least

60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the proteins of Table 4, e.g., 5%-15%, 15%-25%, 25%-35%, 35%-45%, 45%-55%, 55%-65%, 65%-75%, 75%-85%, 85%-95%, or 95%-100% of the proteins of Table 4.

[0429] In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, at least 600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, at least 1000, at least 1050, at least 1100, at least 1150, or all 1195 of the proteins of Table 4, e.g., comprise the extracellular domains of 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1050, 1050-1100, 1100-1150, or all 1195 of the polypeptides of Table 4.

[0430] In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 25% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 50% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 75% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of at least 90% of the proteins of Table 4. In some aspects, the collection of target polypeptides comprises the extracellular domains of all of the proteins of Table 4.

[0431] In some aspects, the extracellular domain of the prey protein (e.g., STM protein) has a native conformation, e.g., a conformation observed in the wild-type protein. In some aspects, the extracellular domain of the prey protein (e.g., STM protein) comprises a native post-translational modification.

[0432] In some aspects, the method further comprises characterizing a cytoplasmic protein profile of the BV (e.g., comprises characterizing proteins present in the lumen of the BV).

[0433] B. Methods of Identifying Changes in Interaction Profiles of Cell Lines

[0434] In some aspects, the disclosure features a method for identifying a change in the interaction profile of a cell line, the method comprising (a) modifying the cell line to comprise a membrane-budding agent; (b) characterizing an interaction profile of a BV produced by the cell line at a first time point; (c) characterizing an interaction profile of a BV produced by the cell line at a second time point; and (d) comparing the interaction profile of the BV produced at the first time point to that of the BV produced at the second time point, wherein a difference between the interaction profile of the BV produced at the first time point and that of the BV produced at the second time point identifies a change in the interaction profile of the cell line.

[0435] In some aspects, the disclosure features a method for identifying a change in the interaction profile of a cell line that has been modified to comprise a membrane-budding agent, the method comprising (a) characterizing an interaction profile of a BV produced by the cell line at a first time point; (b) characterizing an interaction profile of a BV produced by the cell line at a second time point; and (c) comparing the interaction profile of the BV produced at the first time point to that of the BV produced at the second time point, wherein a difference between the interaction profile of the BV produced at the first time point and that of the BV produced at the second time point identifies a change in the interaction profile of the cell line.

[0436] In some aspects, the method comprises exposing the cell line to a stimulus following the first time point and before the second time point; thus, the method can be used to identify changes in the interaction profile of the cell line that occur as a result of exposure to the stimulus. The stimulus may be, e.g., a condition or agent that induces signaling, a condition or agent that induces a disease-related state, and/or a condition or agent that induces differentiation. In some aspects, the cell line is an immune cell line and the disease-related state is immune exhaustion.

[0437] In other aspects, the method does not comprise exposing the cell line to a stimulus. For example, in some aspects, the first time point and the second time point are selected to be at different stages during differentiation of a cell line, or BVs produced at a first time point and a second time point are assessed to determine whether the cell line has differentiated between the time points.

[0438] In some aspects, the method further comprises characterizing an interaction profile of a BV produced by the cell line at one or more additional time points, e.g., at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 additional time points. In some aspects, the method comprises exposing the cell line to a stimulus following the second time point, e.g., exposing the cell line to a stimulus before the one or more additional time points.

[0439] The cell line may be a mammalian cell line. In some aspects, the mammalian cell line is a neuronal cell line, a fibroblast cell line, or an immune cell line. In some aspects, the immune cell line comprises one or more of T-cells, B-cells, or monocytes (e.g., consists of T-cell, or consists of B-cells, or consists of monocytes), e.g., is a T cell line, a B cell line, or a monocyte cell line.

[0440] In some aspects, the cell line is a mammalian cell line representing (e.g., derived from) a tissue type of interest, (e.g., a diseased or healthy tissue type) or a cell type of interest or a cell line representing (e.g., derived from) a tumor of interest (e.g., a tumor cell line). In some aspects, the cell line (e.g., mammalian cell line) is derived from a sample from a subject, e.g., a subject having a disease.

[0441] Exemplary membrane-budding agents are provided in Section III(C) herein. In some aspects, the membrane-budding agent is selected from the group consisting of a HIV gag protein, Acyl.Hrs, ARRDC1, and ARF6. In some aspects, the membrane-budding agent is a HIV gag protein. In some aspects, expression of the membrane-budding agent in the cell line is inducible. Exemplary membrane-budding agents are provided in Section III(C) herein, and exemplary inducible constructs are described in Section VII(A), above. Expression of the membrane-budding agent in the parent cell line, and thus production of BVs by the parent cell line, may be induced at any desired time point. For example, in some aspects, expression of the membrane-budding agent is induced at the first time point, the second time point, and

optionally at one or more additional time points. In some aspects, expression of the membrane-budding agent is not induced in the interval between the first time point and the second time point, e.g., the membrane-budding agent is not expressed in the interval between the first time point and the second time point.

[0442] In some aspects, characterizing the interaction profile of the BV produced by the cell line at a first time point and the BV produced by the cell line at a second time point comprises determining a level of one or more membrane-associated proteins of interest (e.g., one or more receptors of interest) on each of the BVs, as described in Section VII(A), above.

[0443] In some aspects, the method further comprises characterizing a cytoplasmic protein profile of the BV produced by the cell line at a first time point and the BV produced by the cell line at the second time point (e.g., comprises characterizing proteins present in the lumen of the BV produced by the cell line at the first time point and the BV produced by the cell line at the second time point).

[0444] C. Methods of Comparing Interaction Profiles of Cell Lines

[0445] In some aspects, the disclosure features a method for identifying a difference in the interaction profiles of two cell lines, the method comprising (a) modifying each of the cell lines to comprise a membrane-budding agent; (b) characterizing an interaction profile of a BV produced by the first cell line; (c) characterizing an interaction profile of a BV produced by the second cell line; and (d) comparing the interaction profile of the BV produced at the first cell line to that of the BV produced by the second cell line, wherein a difference between the interaction profile of the BV produced by the first cell line and that of the BV produced by the second cell line identifies a difference in the surface protein profiles of two cell lines.

[0446] In some aspects, the disclosure features a method for identifying a difference in the interaction profiles of two cell lines that have been modified to comprise a membrane-budding agent, the method comprising (a) characterizing an interaction profile of a BV produced by the first cell line; (b) characterizing an interaction profile of a BV produced by the second cell line; and (c) comparing the interaction profile of the BV produced at the first cell line to that of the BV produced by the second cell line, wherein a difference between the interaction profile of the BV produced by the first cell line and that of the BV produced by the second cell line identifies a difference in the surface protein profiles of two cell lines.

[0447] In some aspects, the first cell line and the second cell line are mammalian cell lines. In some aspects, the BV produced by the first cell line and the BV produced by the second cell line are derived from different tissues or different cell types. In some aspects, the BV produced by the first cell line is derived from a diseased tissue (e.g., tumor tissue), and the BV produced by the second cell line is derived from healthy tissue.

[0448] Exemplary membrane-budding agents are provided in Section III(C) herein. In some aspects, the membrane-budding agent is selected from the group consisting of a HIV gag protein, Acyl.Hrs, ARRDC1, and ARF6. In some aspects, the membrane-budding agent is a HIV gag protein. In some aspects, expression of the membrane-budding agent in the cell line is inducible. Exemplary membrane-budding agents are provided in Section III(C) herein, and exemplary inducible constructs are described in Section VII(A), above.

[0449] In some aspects, characterizing the interaction profile of the BV produced by the cell line at a first time point and the BV produced by the cell line at a second time point comprises determining a level of one or more membrane-associated proteins of interest (e.g., one or more receptors of interest) on each of the BVs, as described in Section VII(A), above.

[0450] In some aspects, the method further comprises characterizing a cytoplasmic protein profile of the BV produced by the cell line at a first time point and the BV produced by the cell line at the second time point.

[0451] In another aspect, the disclosure features a BV comprising a heterologous membrane-budding agent, wherein the BV is produced by a process comprising (i) providing a parent cell line that has been modified to express the membrane-budding agent under inducible control; (ii) inducing expression of the membrane-budding agent, and (iii) isolating the BV from the parent cell line. In some aspects, the membrane-budding agent is selected from the group consisting of a HIV gag protein, Acyl.Hrs, ARRDC1, and ARF6. In some aspects, the membrane-budding agent is a HIV gag protein. In some aspects, the parent cell line is a mammalian cell line. In some aspects, the BV is an extracellular vesicle (EV).

VIII. Methods of Assessing Membrane Protein Activity Using Biological Vesicles

[0452] In some aspects, the disclosure features a method for assessing an enzymatic activity of a membrane-associated protein, the method comprising conducting an assay for enzymatic activity on a biological vesicle (BV) comprising the protein.

[0453] Exemplary BVs are described in Section III(B) herein. In some aspects, the membrane-associated protein is endogenous to the BV and/or the parent cell thereof, i.e., the enzymatic activity of an endogenous membrane-associated protein is assessed using the method.

[0454] In other aspects, the BV membrane-associated protein is a heterologous membrane-associated protein present on the outer face of the BV, i.e., the enzymatic activity of the heterologous membrane-associated protein is assessed using the method. BVs comprising heterologous membrane-associated proteins are described in Section II herein.

[0455] In some aspects, the heterologous membrane-associated protein is a full-length protein. In other aspects, the heterologous membrane-associated protein is a protein fragment. In some aspects, the heterologous membrane-associated protein comprises a protein fragment, a tag, and an anchor (e.g., an anchor that tethers the protein fragment to the surface of a membrane of the BV, e.g., a glycosylphosphatidyl-inositol (GPI) polypeptide).

[0456] In some aspects, the membrane-associated protein is a peptidase and the assay for enzymatic activity is an assay for peptidase activity, e.g., an assay for degradation of one or more known or putative substrates of the peptidase.

[0457] In some aspects, the membrane-associated protein is a protease and the assay for enzymatic activity is an assay for protease activity, e.g., an assay for degradation of one or more known or putative substrates of the protease.

[0458] In some aspects, the membrane-associated protein is a kinase and the assay for enzymatic activity is an assay

for kinase activity, e.g., an assay for phosphorylation of one or more known or putative substrates of the kinase.

[0459] In some aspects, the membrane-associated protein is a phosphatase and the assay for enzymatic activity is an assay for phosphatase activity, e.g., an assay for dephosphorylation of one or more known or putative substrates of the phosphatase.

IX. Methods of Purifying Biological Vesicles

[0460] In some aspects, the disclosure features a method of purifying a biological vesicle (BV) from a culture medium (e.g., a liquid culture medium) or a sample from a subject (e.g., a liquid sample, e.g., a urine sample, a blood sample, or a digested tissue sample), the method comprising contacting a BV (e.g., a BV in a culture medium) with a solid surface comprising one or more of the generic vesicle binder proteins of Table 8 or Table 9.

TABLE 8

Generic vesicle binder genes
AGER
ALK
AMICA1
APLP2
APP
ASGR1
BTC
C14orf180
C19orf59
CD177
CD22
CD300A
CD300E
CD300LB
CD300LF
CD300LG
CD33
CD6
CD72
CILP
CLEC10A
CLEC14A
CLEC4A
CLEC4G
CLSTN2
CLSTN3
CR2
CSF1R
DDOST
DPCR1
EDA
EPHA6
EPHA7
EPHB1
EPHB2
EPHB3
FCAMR
FCRL2
FGFR4
FGFRL1
FIBCD1
FLT1
FSTL5
GFRA1
GFRA2
GLG1
HAVCR1
HAVCR2
HBEGF
ICAM5
IFNLR1
IGFBPL1
IGSF3
IL15RA
IL1RL1
KLRK1
KREMEN1
KREMEN2
LAG3
LDLR
LILRA1
LILRA3
LILRA6
LILRB2
LILRB5
LRPAP1
MAG
MRC1
MSR1
NFASC
NLGN3
NRG2
NRP1
NRP2
OLR1
PLXDC2
PRND
PRNP
PSG4
PSG5
PTPRB
PTPRD
PTPRF
PTPRS
PXDN
RARRES1
RNF13
ROBO1
ROBO2
RTN4R
SARAF
SELL
SELP
SIGLEC10
SIGLEC14
SIGLEC15
SIGLEC5
SIGLEC6
SIGLEC7
SIGLEC8
SIGLEC9
SIRPA
SIRPG
SPRN
TIE1
TIMD4
TMEM132A
TNFRSF11B
TREML2
WBP1

TABLE 9

List of Generic Vesicle Binder Genes and Scores

Gene Name	Average Score
HAVCR1	1.463141
MAG	1.120588
TIMD4	0.984632
SIGLEC7	0.972398
CD300LF	0.805923
SIRPA	0.76706
SIGLEC9	0.737391

TABLE 9-continued

List of Generic Vesicle Binder Genes and Scores

Gene Name	Average Score
MRC1	0.688295
SIGLEC8	0.674243
CD300LG	0.669373
MSR1	0.572835
SIGLEC10	0.558422
CD22	0.544031
IGSF3	0.533152
SIRPG	0.495548
FCRL2	0.486656
ROBO1	0.463355
SELP	0.458027
CLEC10A	0.426572
C19orf59	0.419371
SIGLEC15	0.414953
TMEM132A	0.413924
FGFRL1	0.401978
ASGR1	0.401957
GFRA1	0.394782
CLEC14A	0.383711
TREML2	0.368332
FLT1	0.364596
PTPRD	0.360015
PTPRB	0.353434
PRND	0.344341
GFRA2	0.339367
NRP1	0.331184
RNF13	0.32993
APLP2	0.320281
SARAF	0.318255
HBEGF	0.309675
EPHB2	0.299617
FSTL5	0.296042
CD300A	0.294351
NRP2	0.283246
LDLR	0.280714
PILRA	0.266112
IFNLR1	0.265823
SIGLEC5	0.264558
NRG2	0.256761
LILRA3	0.251963
EPHB1	0.248776
OLR1	0.246329
CD33	0.240488
CD177	0.240066
CD72	0.23771
LILRB2	0.233592
PRNP	0.232195
RARRES1	0.231731
EPHA6	0.228359
FIBCD1	0.225934
AGER	0.225417
PTPRS	0.221677
ICAM5	0.22125
C14orf180	0.220482
NLGN3	0.21094
BTC	0.202919
PLXDC2	0.199299
EPHB3	0.197941
IL15RA	0.193258
LAG3	0.193037
LILRA1	0.190473
MEGF10	0.189179
ROBO2	0.181367
IL1RL1	0.177439
PSG4	0.169163
LILRB5	0.162532
SIGLEC6	0.156384
LILRA6	0.153831
ALK	0.15204
PSG5	0.147583
CD2	0.137838

TABLE 9-continued

List of Generic Vesicle Binder Genes and Scores

Gene Name	Average Score
PTPRF	0.118992
SORT1	0.110567

[0461] Exemplary BVs are described in Section III(B) herein. The BV may be an unmodified BV or may comprise an exogenous protein (e.g., a heterologous membrane-associated protein, e.g., a full-length heterologous membrane-associated protein or a heterologous membrane-associated protein comprising a protein fragment, a tag, and an anchor) as described in Section II herein. In some aspects, the BV comprises a membrane-budding agent and is produced by a process comprising (i) providing a parent cell that has been modified to express the membrane-budding agent and (ii) isolating the BV from the parent cell.

[0462] The solid surface may be any stationary surface suitable for affinity purification, e.g., a column (e.g., a column comprising Protein A-functionalized beads), a bead, a plane, or a plate.

[0463] The solid surface may be modified to comprise the one or more of the proteins of Table 8 or Table 9 using any appropriate method. In some aspects, the solid surface comprises a moiety with affinity for the one or more proteins of Table 8 or Table 9, and the solid surface is contacted with (e.g., washed with) the one or more proteins of Table 8 or Table 9, thus immobilizing the one or more proteins of Table 8 or Table 9 on the solid surface. The one or more proteins of Table 8 or Table 9 may be modified to comprise a moiety (e.g., a tag), and the affinity of the moiety comprised by the solid surface may be to the moiety or tag. For example, in one aspect, the solid surface comprises Protein A and the one or more proteins of Table 8 or Table 9 have been modified to comprise an Fc region.

[0464] In some aspects, the solid surface comprises a single protein of Table 8 or Table 9. In some aspects, the solid surface comprises HAVCR1, MAG, TIMD4, SIGLEC7, CD300LF, SIRPA, SIGLEC9, MRC1, SIGLEC8, or CD300LG. In some aspects, the solid surface comprises HAVCR1. In some aspects, the solid surface comprises MAG. In some aspects, the solid surface comprises SIGLEC7. In some aspects, the solid surface comprises CD300LF. In some aspects, the solid surface comprises 2, 3, 4, 5, 6, 7, 8, 9, or all 10 of HAVCR1, MAG, TIMD4, SIGLEC7, CD300LF, SIRPA, SIGLEC9, MRC1, SIGLEC8, and CD300LG.

[0465] In some aspects, the solid surface comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more than 25 proteins of Table 8 or Table 9, e.g., comprises 2-5, 5-10, 10-15, 15-20, or 20-25 of the proteins of Table 8 or Table 9. In some aspects, the solid surface comprises two of the proteins of Table 8 or Table 9. In some aspects, the solid surface comprises three of the proteins of Table 8 or Table 9. In some aspects, the solid surface comprises four of the proteins of Table 8 or Table 9. In some aspects, the solid surface comprises five of the proteins of Table 8 or Table 9.

[0466] In some aspects, the one or more of the proteins of Table 8 or Table 9 are human proteins. In some aspects, the BVs are derived from human cells.

[0467] In some aspects, contacting the BV with the solid surface comprises flowing culture medium or a sample from a subject (e.g., a liquid sample, e.g., a urine sample, a blood sample, or a digested tissue sample) comprising the BV over the solid surface. In some aspects, the culture medium is conditioned medium. In some aspects, the culture medium or sample comprises one or more parent cells from which the BV was produced. In other aspects, the culture medium or sample does not comprise parent cells, e.g., has been processed to remove parent cells.

[0468] In some aspects, the method further comprises detaching (e.g., eluting) the BV from the solid surface. BVs may be eluted using any method suitable to detach the BV from the solid surface. For example, washes (e.g., harsh washes, e.g., high-salt washes) and/or appropriate ligands may be used to detach EVs. For example, in some aspects, the one or more proteins of Table 8 or Table 9 comprise a member of the SIGLEC family of proteins and one or more sialoglycans is used in elution.

[0469] In some aspects, the solid surface is a column comprising Protein A-functionalized beads and the method comprises flowing the conditioned media comprising the one or more of the proteins of Table 8 or Table 9 over the column, wherein the one or more proteins of Table 8 or Table 9 have been modified to comprise an Fc region (e.g., a human Fc region), thus immobilizing the one or more proteins of Table 8 or Table 9; flowing the culture medium or sample from the subject comprising the BVs over the column; and eluting the BVs from the column.

[0470] In some aspects, the method comprises an ultracentrifugation-based cleanup step. In other aspects, the method does not comprise ultracentrifugation.

[0471] In some aspects, the method is used for large-scale purification of BVs. In some aspects, the method is performed using a sample volume of at least 10 mL, i.e., at least 10 mL of a culture medium or a sample from a subject is processed according to the method. For example, in some aspects, the method is performed using a sample volume of at least 15 mL, 20 mL, 25 mL, 30 mL, 35 mL, 40 mL, 45 mL, 50 mL, 55 mL, 60 mL, 65 mL, 70 mL, 75 mL, 80 mL, 85 mL, 90 mL, 95 mL, or 100 mL (e.g., is performed using a sample volume of 10-20 mL, 20-30 mL, 30-40 mL, 40-50 mL, 50-60 mL, 60-70 mL, 70-80 mL, 80-90 mL, or 90-100 mL). In some aspects, the method is performed using a sample volume of at least 50 mL. In some aspects, the method is performed using a sample volume of at least 100 mL. For example, in some aspects, the method is performed using a sample volume of at least 150 mL, 200 mL, 250 mL, 300 mL, 350 mL, 400 mL, 450 mL, 500 mL, 550 mL, 600 mL, 650 mL, 700 mL, 750 mL, 800 mL, 850 mL, 900 mL, 950 mL, or 1 L (e.g., is performed using a sample volume of 100-200 mL, 200-300 mL, 300-400 mL, 400-500 mL, 500-600 mL, 600-700 mL, 700-800 mL, 800-900 mL, or 900 mL-1 L).

[0472] BVs purified according to these methods may be used in any of the methods described herein.

[0473] All patent, patent publication and literature references cited in the present specification are hereby incorporated by reference in their entirety.

X. Examples

Example 1. An Extracellular Vesicle-Based Screen for Detection of Extracellular Protein-Protein Interactions in Membranes

[0474] A. Background

[0475] Membrane proteins play an essential role in translating extracellular cues into intracellular responses. Their cell-surface exposure, resulting in increased accessibility to therapeutic molecules, and their ability to orchestrate cellular behavior makes them attractive drug targets. Therefore, it is unsurprising that, while they make up only ~30% of human genes, they account for over 60% of all drug targets (Santos et al., *Nat. Rev. Drug Discov.,* 16: 9-34, 2016.

[0476] The identification of receptor-ligand interactions is thus instrumental to understanding cellular communication occurring in the extracellular milieu. However, progress on the characterization of membrane proteins and their interaction partners has lagged far behind that of cytoplasmic proteins. This is in part due to difficulties in expressing membrane proteins in their native conformations and the lack of techniques that have sufficient sensitivity to detect the weak interactions common for membrane proteins (Martinez-Martin, *J. Immunol. Res,* 2017: 2197615, 2017; Wright, *Mol. Biosyst.* 5: 1405-1412, 2009). Methods designed for general protein-protein interaction discovery require either well-folded purified protein (e.g., microarray- or plate-based screening methods (Martinez-Martin, *J. Immunol. Res,* 2017: 2197615, 2017; Wright et al., *Biochem. Soc. Trans,* 38: 919-922, 2010)), or strong interactions that can survive extraction of proteins from membranes and washes (e.g. affinity purification-mass spectrometry (AP/MS) (Huttlin et al., bioRxiv, doi:10.1101/2020.01.19.905109, 2020)). While some newer approaches have been able to capture many membrane protein interactions by using multimerization to strengthen the weak interactions (Bushell et al., *Genome Res.,* 18: 622-630, 2008; Husain et al., *Mol. Cell. Proteomics,* 18: 2310-2323, 2019), they may still miss key interactions because the proteins are removed from their native membrane contexts.

[0477] Physiological membranes contain a complex mix of lipids, sterols, proteins and glycans that can participate in interactions (Goñi, *Biochim. Biophys. Acta—Biomembr.,* 1838: 1467-1476, 2014). In addition, membranes can strengthen individually weak protein-protein interactions by clustering and orienting (Banjade et al., *Elife,* 3: 1-24, 2014; Taylor et al., *Cell,* 169: 108-119e.20, 2017; Hu et al., *Proc. Nat. Acad. Sci. U.S.A.,* 110: 15283-15288, 2013). These membrane-dependent aspects of the receptor-ligand interactions remain a major bottleneck in the development of screening methods. Recent advances in proximity-based techniques allow detection of interactions in membranes and have been instrumental for the study of transient binders (Geri et al., *Science,* 367: 1091-1097, 2020; Li et al., *Cell,* 180: 373-386.e15, 2020, Gingras et al., *Curr. Opin. Chem. Biol.,* 48: 55-54, 2019). However, these techniques often struggle to distinguish direct interaction partners from nearby bystanders, typically focus on binding partners within the same cell (in-cis interactions), and are often incompatible with high-throughput studies. Alternative approaches such as nanodiscs and liposome particles enable membrane protein reconstitution and have been successfully employed to study challenging receptors (Rouck et al., *FEBS Lett.,* 591: 2057-2088, 2017; De Franceschi et al., *J.*

Cell Sci., 132: 2019). However, these methods require protein purification, which can disrupt native folds and rarely account for potential protein or non-protein cofactors. As a result, the extracellular protein cross-talk remains remarkably underrepresented in existing datasets (Wright et al., *Biochem. Soc. Trans.,* 38: 919-922, 2010; Bausch-Fluck et al., *Proc. Natl. Acad. Sci.,* 2018). These limitations underscore the need for additional techniques specifically designed for the study of membrane proteins, with sufficient throughput and sensitivity for the characterization of receptor interactomes.

[0478] The need for an in-membrane receptor display method has spurred several solutions that take advantage of machinery from enveloped viruses to incorporate receptors into mammalian membranes. For example, microarrays consisting of herpes simplex virions (VirD) displaying different membrane proteins have been used successfully as a GPCR library for ligand discovery (Hu et al., *Proc. Natl. Acad. Sci. U.S.A.,* 110: 15283-15288, 2013; Da Syu et al., *Nat. Commun.,* 10: 1-12, 2019. Additionally, extracellular vesicles containing the HIV gag protein, termed recombinant extracellular vesicles (rEVs), have been used to display multi-transmembrane proteins for immunization and antibody generation (Tucker et al., *Proc. Natl. Acad. Sci. U.S.A.,* 115: E4990-E4999, 2018) as well as antibody selection and ligand-binding characterization (Willis et al., *Biochemistry,* 47: 6988-6990, 2008). These rEVs have lipid and protein compositions similar to the naturally occurring EVs that play a role in cellular communication and pathogenesis of multiple disease (Lavado-Garcia et al., *J. Proteome Res.,* 19: 4516-4532, 2020; Geeurickx et al., *Nat. Commun.,* 10: 1-12, 2019). Therefore, defining interactomes for rEVs can provide insights into both the binding profile of a displayed receptor-of-interest and basic EV biology.

[0479] To address the limitations of these previous approaches, an assay combining the robustness of a direct protein-protein interaction screen with the presentation of a target-of-interest in the context of a membrane was designed using EVs, e.g., rEVs as described above. EVs are nanometer-sized, lipid bilayer-delimited particles that are naturally secreted from cells (Colombo et al., *Annu. Rev. Cell Dev. Biol.,* 30: 255-289, 2014). EVs contain proteins folded and inserted into their native membranes using the cell's endogenous machinery. Herein, EVs are shown to provide a protein-purification free method for obtaining binding-competent receptors. To take advantage of these naturally secreted particles, RDIMIS (Receptor-Display In Membranes Interaction Screen) (also called EVEXIS (extracellular vesicle (EV)-based extracellular interaction screen)), a new platform for receptor-ligand discovery, was developed. RDIMIS allows for rapid, unbiased identification of single-pass transmembrane (STM) protein interactomes by screening any protein-of-interest expressed on EVs against a comprehensive conditioned media library of STM ectodomains (Czajkowsky et al., *EMBO Mol. Med.,* 4:1015-1028, 2012; Martinez-Martin et al., *Cell,* 174: 1158-1171.e19, 2018).

[0480] RDIMIS is a time-efficient method for the elucidation of receptor-ligand interactions that is receptor-agnostic and thus applicable to most targets of interest that can be incorporated in EVs. Profiling the interactome of a membrane protein requires only a plasmid for its expression. The EV context obviates time-consuming and uncertain protein purifications while retaining the advantages of studying proteins in a simplified, plate-based system. EVs use the endogenous cellular machinery to translate, fold and insert the protein into the membrane; therefore, the likelihood of expressing functional protein is optimal. Furthermore, capturing the STM library proteins directly from conditioned media eliminates all protein purification steps, enabling higher throughput studies, and again minimizing resource-consuming purification steps without compromising protein activity (Husain et al., *Mol. Cell. Proteomics,* 18: 2310-2323, 2019). This allows characterization of the interactome of a membrane protein-of-interest to be performed in about a week, limited primarily by the time it takes cells to produce proteins and EVs.

[0481] While the rEV-based approach enables the use of full-length untagged receptor molecules, use of gD-GPI tagged ectodomains can achieve similar results and skirts the need for elusive high-affinity antibodies to measure receptor incorporation. The gD-GPI tag works across receptor families and allows direct quantitative comparison of expression by BLI. This tagging strategy also enables the direct comparison of the interaction profiles of the ectodomain and full-length protein, quickly identifying interactions in which the transmembrane or cytoplasmic domains may play a role. Lastly, the gD-GPI tag can anchor non-membrane proteins, providing a way to study extracellular matrix or secreted factors in proximity to membranes.

[0482] rEVs also contain a cytoplasmic luminal space with cytoskeletal elements that can play roles in higher-order complex formation (Banjade et al., *Elife,* 3: 1-24, 2014; Keerthikumar et al., *J. Mol. Biol.,* 428: 688-692, 2016). In addition to capturing the complexity of cellular membranes, the small size and stability of rEVs (Colombo and Raposo, *Annu. Rev. Cell Dev. Biol.,* 30: 255-289, 2014) make them a very attractive vehicle for high-throughput screening in a physiologically relevant context. As such, the approach implemented in this study can be coupled to any library of choice, from high-coverage collections to more focused libraries such as receptor families or protein disease variants, enabling sensitive and relatively low resource-intensive identification of interactomes while maximizing query protein quality.

[0483] Beyond identification of protein interactomes and deorphanization of complex or hard-to-purify targets, the RDIMIS approach has wide applications in the study of receptor, membrane as well as human EV biology. RDIMIS identified over a hundred vesicle-specific binders for a population of HEK cell-derived, human EVs and showed that the interactome of recombinant and endogenously generated EVs were remarkably similar. This is consistent with previous works showing strong similarities between the proteomic and lipid compositions of rEVs and endogenous EVs (Lavado-Garcia et al., *J. Proteome Res.,* 19: 4516-4532, 2020; Geeurickx et al., *Nat. Commun.,* 10: 1-12, 2019). Therefore, these binders may shed light on endogenous EV tropism and signaling. Results indicate that EVs interact with major signaling proteins such as immunomodulatory proteins (i.e. SIGLEC family, LILR family and CD300 family), growth regulators proteins (i.e. growth factor receptors like FGFR4, FLT1 and NRP proteins and several receptor-tyrosine kinases) or neuronal proteins (i.e. APP and CLSTN proteins), supporting the idea that EVs mediate intercellular communication. This work sets the stage for future studies focused on disease-, tissue- or cell-specific EVs and their roles in cellular communication or immune

responses. Given the throughput and reproducibility, this method provides a way to assess the quality of EVs on the global scale. As EVs are being explored as avenues for drug delivery (Vader et al., *Adv. Drug Deliv. Rev,* 106: 148-156, 2016), RDIMIS can provide a way to address concerns about variability, immunogenicity and off-target effects associated with EV complexity. Since this platform provides a unique tool to elucidate the players that influence EV functions at the molecular level, it can detect unexpected changes in the binding profile of EVs arising from the overexpression of target receptors, addition of therapeutics or modifications of the cells generating the EVs.

[0484] B. Extracellular Vesicles for Membrane Display

[0485] A method for receptor-ligand discovery in native membranes requires several parts. First, it must capture stable pieces of cellular membranes. This can be achieved by purifying EVs, lipid bilayer-contained particles naturally generated by most cells (FIGS. **1**A-**1**C) (Colombo et al., *Annu. Rev. Cell Dev. Biol.,* 30: 255-289, 2014). EVs incorporate a host of membrane-associated macromolecules (Keerthikumar et al., *J. Mol. Biol.,* 428: 688-692, 2016), making them a microcosm of the cellular membrane environment (FIG. **2**A). This environment often participates in membrane protein interactions, making EVs a very attractive basis for a high-throughput platform.

[0486] Second, large quantities of EVs are likely necessary for high-throughput, sensitive screening. In order to maximize EV production, EXPI293F™ cells, which provide an efficient system for protein expression, were chosen (Heath et al., *Sci. Rep.,* 8: 1-12, 2008; Arena et al., *MAbs,* 11: 977-986, 2019). EXP1293F™ cells were cultured in EXP1293™ Expression Media, shaking, at 150 rpm. All cells were cultured at 37° C., 5% CO_2.

[0487] To further boost EV production (Geeurickx et al., *Nat. Commun.,* 10: 1-12, 2019; Cervera et al., *J.* Biotechnol., 166: 152-165, 2013), cells were transfected with an HIV gag construct (FIG. **2**B). Gag expression increased the yield of 20-500 nm vesicles by almost 4-fold (FIG. **2**C).

[0488] Third, robust and quantitative methods are needed for detecting EVs. Cells release their EVs into a surrounding medium that also contains a complex and variable background of other molecules. These make it challenging to get reproducible quantitative data from downstream assays. To address this issue, a one-step density-based EV purification was optimized. This gave a pure and consistent population of rEVs free of aggregates (FIGS. **1**A and **1**B). Lastly, high-throughput screening necessarily shrinks the scale of each reaction. This creates a challenge for robust and quantitative detection of bound EVs. *Renilla* luciferase (Rluc) was therefore fused to the HIV gag construct. This gave robust luminescence signals linearly proportional to vesicle concentration over nearly three orders of magnitude (FIG. **2**D).

[0489] Next, to determine whether EVs are broadly applicable as platforms for receptor display, a variety of full-length, untagged, receptors were overexpressed in cells along with gag-Rluc. In all cases tested, the receptors were readily detectable in EVs using antibodies specific for the receptors (FIG. **4**A). To test a larger set of receptors and measure their incorporation in EVs, detection methods not dependent on receptor-specific antibodies were needed. To address this, a collection of unrelated receptor ectodomains was fused to a glycoprotein D (gD)-glycosylphosphatidylinositol (GPI) tag. The GPI provides a lipid anchor that maintains ectodomains in membranes, while the gD epitope tag allows ectodomain detection using an anti-gD antibody. When these receptors were tested for incorporation into vesicles, most of the receptors assayed were readily detectable in EVs using an anti-gD antibody (FIGS. **4**B, **4**C, and **13**A). Thus, the gD-GPI tagging strategy allows for easy detection of a variety of proteins in EVs.

[0490] C. Generation of Extracellular Vesicles Expressing Membrane Proteins

[0491] HEK293T or EXP1293™ cells were transiently transfected with a receptor of interest or an empty vector control (as indicated) and a plasmid expressing HIV gag fused to either Rluc (for screening) or mNeonGreen (for visualization). All plasmids transfected were cloned into the expression vector pRK5 (Genentech). For vesicle harvesting, cells were removed by either spinning at 300×g for 10 minutes followed by clearing at 2000×g for 20 minutes (for expressions <100 mL) or filtered out (for expressions ≥100 mL). For full library screens, rEV expression was done at a 1 L scale and grown for 7 days. cOmplete™ EDTA-free Protease Inhibitor Cocktail Tablets (Roche) were added as per specifications. After fully dissolving the tablets, the conditioned media was spun at 12,000×g for 40 minutes to remove any remaining dense particulates and microvesicles. The supernatants were transferred to 70 mL polycarbonate ultracentrifuge tubes (Beckman Coulter) at a volume of about 60 mL per tube. 10 mL of 50% sucrose were layered from the bottom of the tube using a syringe and long needle, forming a sucrose cushion. Samples were spun at 100,000×g for 90 minutes in a Ti-45 rotor. Vesicles float on top of the sucrose. Media above the vesicle layer was aspirated. Two tubes worth of cushion and vesicles were combined and diluted to 70 mL in new ultracentrifuge tubes using PBS. Samples were spun again at 100,000×g and the resulting pellet was dissolved in PBS. HALT™ Protease Inhibitor Cocktail (Thermo Fisher Scientific) was added to 1×. All spins were done at 4° C.

[0492] HEK293T cells were used to confirm that overexpressing receptors and gag protein fusions were detectable in vesicles. HEK293T were cultured in DMEM+GlutaMax supplemented with 10% FBS and penicillin/streptomycin (Thermo Fisher Scientific).

Example 2. Identification of Binding Partners for PVR

[0493] A. Identification of Binding Partners for PVR Using EVs

[0494] To determine whether receptors expressed on EVs were accessible and binding competent, a proof-of-concept study was performed using the receptor PVR.

[0495] The identification of binding partners for a receptor-of-interest requires that the rEVs display accessible and binding-competent receptor on their surfaces. The poliovirus receptor (PVR) was used for proof-of-concept studies. PVR is a useful benchmark because it is known to bind to a variety of well-characterized cell surface-expressed receptors with different binding affinities (Husain et al., *Mol. Cell. Proteomics,* 18: 2310-2323, 2019). To determine whether PVR on rEVs was active, rEVs expressing full-length PVR were isolated and tested for binding to a number of PVR binding partners, expressed on the cell surface. PVR-rEVs selectively bound to cells expressing PVR ligands, with negligible background in untransfected cells under the conditions tested (FIG. **2**E).

[0496] Next, to determine whether the gD-GPI strategy resulted in binding-competent receptor display, the PVR ectodomain was fused to a gD-GPI tag. gD-GPI vesicles were measured at a total protein concentration of 0.1 mg/mL in PBS against 10 μg/mL mouse anti-gD antibody (Abcam) using Anti-Mouse IgG Fc Capture Biosensors (ForteBio) by BLI. Similar to EVs expressing full-length PVR, PVR ectodomain-gD-GPI EVs selectively bound to cells expressing the PVR ligands, indicating that the tagged ectodomain was active for binding (FIG. **1**F). The gD tag also enabled detection of PVR receptor expression on the EVs by electron microscopy (FIG. **4**C). For general vesicle staining, the suspension of vesicles was adsorbed for 15 minutes to the surface of formvar- and carbon-coated TEM grids. After a short rinse with distilled water, samples were stained with 2% phosphotungstic acid (PTA) for 60 seconds and then air dried. For gD epitope detection, vesicles were adsorbed for 30 minutes and blocked with Aurion Blocking Solution for goat conjugates for 30 minutes. Samples were then stained with mouse anti-gD (abcam) for 1 hour in the blocking solution and detected using goat-anti mouse 12 nm gold conjugate. Samples were then washed with PBS for 15 minutes, washed with water for 1 minute, and stained with 1% uranyl acetate for 1 minute before being blotted and air dried. Imaging was done with a JEOL JEM-1400 transmission electron microscope (TEM) and a GATAN ULTRASCAN® 1000 CCD camera at magnifications from 5,000× to 50,000×.

[0497] B. Comparison of EV-Based Assays to Assays Using Recombinant Protein

[0498] Next, we determined whether rEVs can bind tightly enough to survive the washing steps and handling delays of our high-throughput assays. To this end, we determined the kinetics of binding for rEVs displaying either full-length PVR or the gD-GPI tagged ectodomain of PVR and for recombinant monomeric PVR to the known ligand CD226. Using a non-destructive technique, biolayer interferometry (BLI), a strong negative BLI signal was observed for both PVR rEV types, as expected given the large size of the rEVs (FIG. **2**G, left panel). This was not observed when PVR-rEVs were incubated with a human IgG as a control, suggesting that this is a specific interaction (FIG. **2**G, right panel). The rEVs showed little dissociation over ten minutes, making them suitable for high-throughput screening (FIG. **2**G, right panel). Further, the EVs achieved faster association and slower dissociation relative to the PVR monomer, indicative of a higher affinity interaction (FIG. **2**G, right panel). Notably, and consistent with the cell-based assays (FIGS. **2**E and **2**F), the gD-GPI tagged PVR EVs showed a similar binding profile to the full-length PVR EVs, further showing that the engineered receptor is amenable for detection of in-trans binding partners.

[0499] C. BLI Methods

[0500] BLI measurements were performed using an 8-channel OCTET® RED system (ForteBio). For PVR rEV binding, CD226-Fc (R&D SYSTEMS®) or native human IgG (abcam) was loaded at 25 nM onto Anti-Human IgG Fc Capture Biosensors (ForteBio). All recombinant proteins were loaded for 300 seconds. All measurements were performed at 30° C. Analysis was performed on the Octet® System Data Analysis software (ForteBio). A PBS buffer control was subtracted to account for drift in the instrument (unless PBS curve is shown). Alignment was to baseline and Savitzky-Golay filtering was performed. Values greater than 2 nm of association over 600 seconds are recommended.

[0501] D. EV Sample Characterization Methods

[0502] Total protein concentrations of the EV samples were measured by mixing 1.5 μL of the sample with 148.5 μL of Quick Start Bradford Protein Assay Reagent (Bio-Rad). The concentration was calculated against a titrated BSA curve. This reagent contains methanol, which permeabilizes membranes, so no detergents were added. EV particle numbers and concentrations were calculated from nanoparticle tracking analysis using NanoSight NTA (Malvern Panalytical). Vesicles at 0.1 mg/mL total protein were diluted 1000× in PBS and run for 5 repeats of 1-minute-long recordings using a 488 nm laser. Traces were analyzed using NTA 3.4 software, which provided a particle concentration that was used to calculate a molarity.

[0503] E. Discussion

[0504] This example demonstrates that both full-length, native proteins and gD-GPI tagged ectodomains of the receptors under study are incorporated in EVs and are amenable to EV-based binding studies. Anchoring ectodomains to EVs using gD-GPI tagging increases the capabilities of RDIMIS. First, it enables comparison of the interaction profiles of ectodomains and full-length proteins. Second, when studying unrelated receptors, the gD-GPI tag provides a common way to compare expression and localization across receptors, avoiding the need for often unavailable receptor-specific antibodies. Lastly, the gD-GPI tag can anchor non-membrane proteins, providing a way to study extracellular matrix or secreted factors in proximity to membranes.

[0505] Levels of the target of interest in EVs appear to depend primarily on the level of expression in parent cells. Therefore, for low-expressing proteins, optimization of experimental conditions may be required to achieve sufficient expression for efficient detection of binding partners.

Example 3. RDIMIS Enables Identification of STM Protein Interactomes in High Throughput

[0506] A. Design of EVs and Ectodomain Library for High-Throughput Screen

[0507] The RDIMIS system was next used for high-throughput discovery of receptor-ligand interactions (FIG. **3**A).

[0508] To achieve unbiased identification, EVs expressing a protein of interest were assessed for binding with a previously developed conditioned media library consisting of most human single-pass transmembrane (STM) proteins expressed as ectodomain-Fc tag fusions (Martinez-Martin et al., *Cell,* 174(5): 1158-1171, 2018). To generate the library, cells were transfected with plasmids encoding the ectodomain-Fc tag fusions to induce them to express and secrete Fc-tagged ectodomains into the growth media. The media was then transferred to protein A-coated plates, with each well of the plate receiving a different Fc-tagged ectodomain. This resulted in a collection of immobilized ectodomains suitable for high throughput screening (Martinez-Martin et al., *Cell,* 174(5): 1158-1171, 2018).

[0509] In parallel, cell cultures for EV production were transiently transfected with plasmids encoding the receptor of interest and gag-*Renilla* luciferase (Rluc) (FIG. **3**A). The receptor-containing EVs were isolated using an optimized purification protocol that enabled rapid large-scale isolation of EVs (FIG. **1**A). After EVs were isolated, they were

incubated with the plates containing the STM protein library. Plates were then thoroughly washed to remove unbound EVs. To detect interactions between the receptor-containing EVs and the STM proteins immobilized in the wells, Rluc substrate was added, thus generating a fluorescent signal in the wells where an interaction had taken place (FIG. **3**A).

[0510] B. STM Interactome of PVR EVs

[0511] The RDIMIS platform was used to study the STM interactome of PVR-EVs (isolated EVs carrying a gD-GPI-tagged PVR ectodomain). Display of the receptor on the vesicles was confirmed using BLI and by Western blot, which showed robust binding of the rEVs to the gD antibody (FIGS. **4**D and **13**A). Notably, RDIMIS identified all expected PVR binding partners: CD96, CD226, KIR2DL5A, PVRL3, PVRL4, and TIGIT (FIG. **3**B, blue) reproducibly across two independent rEV and STM library preparations (correlation coefficient of 0.90; FIG. **16**A). These screens were performed using two independent PVR-EV preparations and two independent STM library preparations to control for potential variability during expression or preparation of the samples. Moreover, and importantly, the hits were virtually identical when RDIMIS was utilized to study rEVs carrying full-length, untagged, PVR (FIG. **3**C), with an overall correlation coefficient of 0.88 (FIG. **16**A). Together, these results further demonstrate that the expression of gD-GPI tagged receptors on rEVs allows detection of relevant ligands in-trans, which, in combination with the automated workflow developed, enables robust identification of membrane protein interactomes in an unbiased fashion and with enhanced sensitivity for detection of high and low affinity interactions.

[0512] C. STM Interactome of B7 Family Proteins

[0513] To further benchmark the sensitivity of this technique, the platform was applied to study three members of the B7 family of immunoregulatory proteins, which contains prominent immune receptors including the checkpoint inhibitor PD-L1. All three proteins were expressed as gD-GPI ectodomain fusions, allowing for their expression in vesicles to be monitored and directly compared with PVR gD-GPI containing rEVs. In all rEVs, the tagged ectodomains bound to an anti-gD antibody at levels comparable to PVR when assayed by BLI (FIG. **4**D) or by Western blot (FIG. **13**A).

[0514] a. STM Interactome of PD-L1 EVs

[0515] RDIMIS was applied to, PD-L1/CD274. PD-L1 was expressed as a gD-GPI-tagged ectodomain to allow for characterization of the PD-L1 EVs prior to screening, as described above. The PD-L1 EVs showed readily detectable binding to the gD antibody by BLI (FIG. **4**D). When screened for interaction with the STM library, PD-L1 EVs identified the known PD-L1 ligands PDCD1 (PD1), EPHA3, CD80 (B7.1), and PDCD1 LG2 (PDL2) with high confidence. Since the estimated dissociation constant for the PD-L1/PD-L2 interaction is approximately 10 μM (Lee et al., *Nat. Commun.,* 7: 1-9, 2016), this result further demonstrates that RDIMIS can identify biochemically challenging weak interactions. In addition, a number of other high-scoring hits were identified (FIG. **5**A). While IGF2R has been found to be broadly sticky in unrelated experiments (Husain et al., *Mol. Cell. Proteomics,* 18: 2310-2323, 2019) and was thus labeled as non-specific interactor, the other hits represent new putative binders for PD-L1.

[0516] b. STM Interactome of CD80 and CD276 EVs

[0517] Finally, to further ensure the wide applicability of RDIMIS, the newly platform was applied to two additional membrane-expressed receptors, CD80 (B7-1) and CD276 (B7-H3) (FIG. **5**B). Again, all relevant partners were detected for both proteins, confirming the broad utility of this methodology to cell surface-expressed targets. The well-described binders CD28, CTLA4, and PD-L1 and the more recently described binder NGFR were identified as the highest-scoring hits for the immune receptor CD80. In the case of B7-H3/CD276, deorphanized only recently through advances in screening technology (Husain et al., *Mol. Cell. Proteomics,* 18: 2310-2323, 2019), RDIMIS captured the recently described interactor IL20RA, as well as MXRA5, which was found to be a non-specific interactor in the same study (FIG. **5**B). Notably, in both cases, the known interaction partners were among the highest-scoring hits, while several additional putative receptor-specific binding partners were identified that were previously not described in the literature, demonstrating the sensitivity of this method.

[0518] D. Comparison with Published Databases

[0519] Next, to get an overall sense of the number of the landscape of interactions identified for these four prominent immune receptors, the overlap between the receptor-specific hits identified using the RDIMIS method and interactions listed in the STRING (Szklarczyk et al., *Nucleic Acids Res.,* 47: D607-D613, 2019), Bioplex (Huttlin et al., bioRxiv, doi:10.1101/2020.01.19.905109, 2020) and Biogrid (Oughtred et al., *Nucleic Acids Res.,* 47: D529-D541, 2019) databases (FIG. **5**C), some of the most comprehensive repositories for protein interactions, was assessed.

[0520] For an even comparison, only interactions between proteins that were present in the STM library queried in this study were considered. For the STRING database, only interactions designated as having experimental evidence were used. To generate a stringent list of hits for all screens, a cutoff was drawn at the 98% quantile for each screen because the distribution of the data deviated from a normal distribution and had a long upper tail. In all screens, a receptor-specific hit was called if signal in a particular screen was at least 4× that of the other screen.

[0521] As expected, most of the well-characterized interactions were represented in at least one of the databases, with eleven out of twelve interactions in STRING listed as having experimental evidence overlapping with our dataset (FIG. **5**C). In addition, for all immune receptors under study, RDIMIS identified putative interactions that were not represented in publicly available databases (FIG. **5**C). This suggests that identification of new interactions may be facilitated when the receptors of interest are studied in the context of the plasma membrane.

[0522] For CD80, the STRING database suggested an additional binding partner, CD86. Additional review of the literature, including the paper cited in support of this interaction in the STRING database, suggested these proteins do not appear to interact. While Bioplex and Biogrid do identify additional putative partners, these have largely not been validated.

[0523] E. RDIMIS Screen Methods

[0524] Vesicles were diluted into a final concentration of 0.03-0.05 mg/mL (as measured by Bradford) in 1×PBS+0.49 mM MgCl2+0.9 mM $CaCl_2$) (PCM)+1% BSA Fraction V (Sigma). Preparation of the human receptor library was performed using an integrated robotic system consisting of automated liquid handling devices (plate dispensers and

washer) to allow for high throughput analysis of protein-protein interactions. Conditioned media containing Fc-tagged receptor ECDs were dispensed into white 384 well Protein A-coated plates (Thermo Fisher Scientific) and stored at 4° C. until needed. Concentration of the ECD-Fcs, varied but averaged 159 μg/mL. Plates were washed three times with PCM to remove unbound components of the conditioned media. Vesicles were added to the plates and allowed to sit overnight at 4° C. Plates were washed three times with PCM to remove unbound vesicles. To prevent drying, 25 μL of PCM was added to the plates. For a positive control used for normalization, 25 μL of the same vesicle stocks used in the screens were added into the first column of each plate after all washing steps. Since these are not washed, these positive control wells represent an input value, though due to automation limitations, they are diluted when compared to a well that is 100% bound. 25 μL of 1 μM coelanterazine h (Promega) in PCM is dispensed into the wells, incubated for 5 minutes, and then read on a TECAN using 0.1 s of luminescence read time. Since the majority of wells in the screens did not bind to any of the vesicles, additional negative control wells were not used in the analysis; rather, signals were analyzed with respect to the distribution of intensities across the whole screen to call hits. Empty wells tended to have higher signal than wells that had received ECD-Fc conditioned media that fell into the bottom half of the distribution and did not represent a non-binding Fc signal.

[0525] F. Quantification of EVs Using a Membrane Stain

[0526] Cholesterol is a main component of the lipidic bilayer of the EVs. EVs were labeled with the AMPLEX™ Red Cholesterol Assay Kit (Thermo Fisher), thus enabling detection of EVs comprising non-tagged targets and/or endogenous EVs. A full RDIMIS screen was performed using either rEVs that were transfected with a vector control but contained gag-Rluc or EVs harvested from untransfected cells (FIG. **12**C). Both EV species behaved similarly (correlation coefficient of 0.92), showing that rEVs and naturally generated EVs from the same cell line have a similar binding profile.

[0527] EVs were generated and harvested as described above. PD-L1 or PVR gD-GPI EVs containing gag-Rluc were serially diluted into a white 384 well plate, and EV concentration was measured using either Rluc by the addition of coelenterazine h (as in RDIMIS) or using the AMPLEX™ Red Cholesterol Assay Kit (Thermo Fisher) following the recommended protocol. To measure PD-L1 gD-GPI RDIMIS using the AMPLEX™ Red Cholesterol Assay Kit, EVs were allowed to bind as with normal RDIMIS. Plates were then aspirated dry, and 25 μL of the AMPLEX™ Red reagent was dispensed into each well. Stock EV solution was added to the first column of each plate to serve as a positive control. Plates were incubated for 30 minutes at 37° C. and read on a Tecan M1000 InfinitePro using excitation at 560+/1 10 nm and fluorescence detection at 590+/−10 nm (FIGS. **12**A-**12**C).

[0528] Cholesterol esterase was included at the recommended amounts to ensure all cholesterol esters were also detected. Vesicles were incubated with Fc-ECD proteins as in the EVEXIS screen and washed as with EVEXIS. Rather than adding coelantrazine-h, plates were manually flicked dry. For the titration against luciferase signal, four 3× serial dilutions were made. 20 μL of 0.5 μM Coelantrazine h (Promega) was added, incubated for 5 minutes, and read on a Tecan M1000 InfinitePro. 20 μL of the Amplex Red Cholesterol Assay mix, as specified in the manual, was added to the wells, incubated for 1 hour, and read. Luminescence was read out on a Tecan M1000 InfinitePro using 0.1s of luminescence read time. Fluorescence was read out in a TECAN using an excitation of 560 nm and an emission wavelength of 590 nm. A blank well with just PBS and Coelantrazine h and Amplex Red Cholesterol Assay mix was also measured and the values subtracted from the signal. For PD-L1 gD-GPI EV binding, wells were flicked dry between the Coelantrazine h and Amplex Red Cholesterol Assay. Rather than subtracting a blank well, a well where no Fc-tagged species was transfected but conditioned media was still added, was used.

[0529] G. Interactions of LRTM1

[0530] To further assess the sensitivity of the assay, one of the new hits that had a low signal, LRTM1, was investigated. Since commercially available protein could not be found for LRTM1, LRTM1 expressed on rEVs was used to study the interaction. This study showed that PD-L1 ectodomains selectively bound to LRTM1 on rEVs presented as either a gD-GPI tagged ecotodomain or as the full-length protein (FIG. **13**B). LRTM1 rEVs also selectively bound to gD-GPI tagged or full-length PD-L1 expressed on cells over cells transfected with a vector control (FIG. **13**C). Since PD1 (PDCD1) is a known interaction partner of PD-L1 and the target of checkpoint blockade immunotherapy in cancer, experiments were performed to determine whether these interactions competed. Increasing concentrations of recombinant PD1-Fc protein were able to outcompete LRTM1 vesicle binding in a concentration-dependent manner (FIG. **13**D), suggesting that the proteins bind to similar regions on PD-L1.

Example 4. RDIMIS Enables Identification of Generic Extracellular Vesicle Binders

[0531] Interestingly, all unrelated screens revealed additional hits scattered among the expected binding partners (Table 8). As rEVs are complex mixtures, it was not clear whether these new binders were specific to the receptors expressed on the rEVs, or to the vesicles themselves. To address this, screens for rEVs displaying different receptors were directly compared to identify common and differential binders. Batch-matched screens were plotted against each other (FIGS. **5**A and **5**B) or against a screen with cells transfected with a vector control instead of a receptor-of-interest done independently (FIGS. **14**A-**14**F). This revealed three distinct groups: PVR-specific binders (blue), PD-L1-specific binders (red), and a population of hits that were not enriched in a particular screen and therefore, were not receptor-specific (FIGS. **5**A and **5**B, gray shading; Tables 8 and 9).

[0532] Notably, several protein families were enriched in the generic vesicle binder list, including sugar binders like CLECs and SIGLECs, but also signaling receptors like LILRs and ephrin receptors. To determine whether any biological pathways or functions were overrepresented, gene ontology (GO) enrichment analysis was performed for the set of generic vesicle binders identified in the four RDIMIS screens shown in FIGS. **5**A and **5**B. Table 10 shows results of a GO enrichment analysis for the generic vesicle binders of Table 8, as performed using PANTHER15.0 overrepresentation test (2020 Mar. 23 release). Table 11 shows results of a GO enrichment analysis for the generic vesicle binders

of Table 9, as performed using the PANTHER16.0 release overrepresentation test (Mi et al., *Nucleic Acids Res,* 49: D394-D403, 2021). The molecular functions significantly enriched included carbohydrate, sulfur and anion binding, all consistent with general binding to vesicles and cellular membranes. The list was also enriched in proteins associated with tertiary granule and tertiary granule membranes by a GO cellular component analysis (Table 12), further suggesting that these are general EV binders. GO biological process analysis was also performed but no significant results were found. Thus, this platform enables the identification of vesicle-specific binders that are conserved across interactomes for unrelated targets of interest, suggesting previously unknown receptors for the EVs.

TABLE 10

GO enrichment analysis for generic vesicle binders

GO molecular function	raw P-value	FDR
carbohydrate derivative binding	9.40E−09	1.02E−05
anion binding	1.82E−07	9.88E−05
sialic acid binding	3.65E−07	1.32E−04
heparin binding	2.59E−06	7.02E−04
sulfur compound binding	6.37E−06	1.38E−03
carbohydrate binding	6.48E−06	1.17E−03
glycosaminoglycan binding	6.14E−05	9.50E−03
carboxylic acid binding	8.90E−05	1.20E−02
organic acid binding	8.90E−05	1.07E−02
axon guidance receptor activity	1.47E−04	1.59E−02
small molecule binding	2.09E−04	2.06E−02

FDR: false discovery rate.

TABLE 11

GO enrichment analysis for generic vesicle binders

GO molecular function	raw P-value	FDR
carbohydrate derivative binding	1.20E−07	1.29E−04
sialic acid binding	2.10E−07	1.13E−04
heparin binding	6.57E−06	2.36E−03
sulfur compound binding	1.45E−05	2.60E−03
carbohydrate binding	8.41E−06	.26E−03
glycosaminoglycan binding	7.87E−09	1.06E−02
carboxylic acid binding	8.63E−06	1.86E−03
axon guidance receptor activity	3.01E−04	2.94E−02
transmembrane signaling receptor activity	6.67E−05	1.03E−02
signaling receptor activity	7.89E−05	9.44E−03
molecular transducer activity	7.89E−05	8.50E−03

FDR: false discovery rate.

TABLE 12

GO cellular component analysis for generic vesicle binders

GO cellular component	raw P-value	FDR
tertiary granule membrane	1.80E−05	1.28E−02
tertiary granule	7.25E−05	2.58E−02
cytoplasmic vesicle	2.38E−04	5.65E−02
intracellular vesicle	2.46E−04	4.38E−02

[0533] While the GO analysis suggested that many of the generic vesicle binders recognize common cell-surface modifications, many of the binders may also have protein interaction partners in the rEVs. To generate a list of potential binders, the generic vesicle binder list was cross-references with the published immunoglobulin superfamily receptome (Verschueren et al., *Cell,* 182: 329-344.e19, 2020) and the STRING database of interactions (Szklarczyk et al., *Nucleic Acids Res.,* 47: D607-D613, 2019). Using these data, a network of potential interactions was generated (FIG. **15**). Each node is a protein found in the network color-coded by whether it is a generic vesicle binder (green) or one identified from the published works and databases (blue). The source of the data is color-coded in the edges. To get a sense of whether these proteins are likely to be in the vesicles, expression data were cross-referenced for HEK293 cells, the parent cell line for the EXPI293™ cells used to generate the rEVs, using data from the Cell Atlas (Thul et al., *Science,* 356(6340), 2017. Based on the finding that the incorporation of a protein into the rEVs was typically correlated with its expression in the cells, it was reasoned that a highly expressed protein was more likely to be responsible for the binding that was detected. Expression is shown as the height of the boxes surrounding each protein name in FIG. **15**, with taller boxes representing greater expression. This network suggests several highly expressed proteins that have known interactions with proteins in the generic vesicle binders list.

[0534] While high reproducibility was found for screens performed at the same time (PVR gD-GPI repeat 2 and PD-L1 gD-GPI; CD276 and CD80 gD-GPI; LRRC15 and PVR FL) or between cells expressing the same receptor (PVR gD-GPI repeats or PVR-FL), some variability was observed when all of the screens were plotted against each other (FIG. **16**A). In particular, while some of the lower correlation was driven by receptor-specific hits (i.e. PVR gD-GPI vs. PD-L1 gD-GPI), the screens with the worst correlation were the CD80 gD-GPI and PVR FL screens. Interestingly, that this was driven by two separable populations of generic vesicle binders, one that seemed to be consistent between the screens (FIG. **16**B, sky blue dots) and one that was enriched in the CD80 gD-GPI screen (FIG. **16**B, gold dots). However, both populations could still be distinguished from CD80 gD-GPI specific hits, as those hits were significantly more enriched in the CD80 gD-GPI screen. This could be seen by either zooming in near the y-axis (FIG. **161***a*) or by removing the list of generic vesicle binders common between all of the screens (FIG. **1-60**). To determine whether was some difference between these two populations, GO molecular functions analysis was performed on the two populations identified in this screen (Tables 13 and 14). Interestingly, heparin and glycosaminoglycan binding was enriched in the first population of generic vesicle binders (Table 13), while sialic acid binding and transmembrane signaling activity was enriched in the second population (Table 14).

TABLE 13

CD80 gD-GPI enriched generic binders

GO Molecular Function	Raw p-value	FDR
Heparin binding	4.52E−08	4.87E−05
Sulfur compound binding	1.42E−07	7.62E−05
Glycosaminoglycan binding	3.78E−06	1.36E−03
Carbohydrate derivative binding	1.75E−04	4.71E−04

TABLE 14

PVR FL enriched generic binders

GO Molecular Function	Raw p-value	FDR
Sialic acid binding	4.58E−07	4.93E−04
Anion binding	3.98E−06	2.14E−03
Transmembrane-ephrin receptor activity	5.21E−06	1.87E−03
Carbohydrate derivative binding	5.90E−06	1.59E−03
Ephrin receptor activity	9.69E−06	2.09E−03
Carboxylic acid binding	1.10E−05	1.98E−03
Carbohydrate binding	5.29E−05	8.14E−03
Transmembrane receptor protein tyrosine kinase activity	3.64E−04	4.90E−02

[0535] To get the list for each individual pair of screens, a cutoff was drawn at the 90% quantile because the distribution of the data deviated from a normal distribution and had a long upper tail. This was done for each screen individually and the final list was the list of genes that was common between all screens which removed screen-specific hits. To rank the genes, the intensity was averaged across all screens and sorted by that average. Gene Ontology analysis was done using the PANTHER15.0 release overrepresentation test (2020 Mar. 23 release) or the PANTHER16.0 release overrepresentation test (Mi et al., *Nucleic Acids Res,* 49: D394-D403, 2021) on the list of generic vesicle binders. The reference list was a list of all genes in the STM library. GO molecular function complete, biological process complete and cellular component complete annotation data sets were analyzed using the Fisher's exact test.

[0536] The RDIMIS approach thus allows profiling of vesicle-specific binders, e.g., for the study of human EV biology. More than 100 binders for HEK cell-derived, human EVs were identified, suggesting putative receptors that may mediate EV interactions with cells, an aspect of vesicle biology that has remained elusive due to the lack of optimal tools (Gonda et al., *Mol. Cancer Res.,* 17: 337-347, 2019). Our results indicate that EVs interact with major signaling proteins such as immunomodulatory proteins (e.g. SIGLEC family, LILR family and CD300 family), growth regulator proteins (e.g. growth factor receptors like FGFR4, FLT1 and NRP proteins and several receptor-tyrosine kinases) and neuronal proteins (e.g. APP and CLSTN proteins), supporting the idea that EVs mediate intercellular communication. Given the throughput and reproducibility, this method provides a way to assess the quality of EVs on the global scale. Further, this platform provides a unique tool to elucidate the players that influence EV functions at the molecular level; therefore, it can be used to detect unexpected changes in the binding profile of EVs arising from the overexpression of target receptors, addition of therapeutics, or modifications of the cells generating the EVs.

Example 5. RDIMIS Identifies CD248 as a Novel Interaction Partner for the Orphan Cancer-Relevant Receptor LRRC15

[0537] The above-described results suggested that RDIMIS may enable deorphanization of challenging targets refractory to other biochemical screening approaches. As an example, RDIMIS was used to study the cancer-associated fibroblast (CAF) receptor LRRC15. LRRC15 recently emerged as a specific marker for CAFs associated with large tumors (Dominguez et al., *Cancer Discov.,* 10(2): 232-253, 2020). Despite this biological importance, no interaction partners have been identified, and thus basic aspects of LRRC15 biology remain undefined. LRRC15 interaction partners were first searched for using a previously implemented technology, miniaturized AVEXIS (Martinez-Martin et al., *Cell,* 174(5): 1158-1171, 2018; Bushell et al., *Genome Res.,* 18: 622-630, 2008). This technology screens pentamerized LRRC15 ectodomain against the STM protein library described above. Despite the high sensitivity of the AVEXIS screen for detection of transient interactions previously shown with this technology, no binding partners were identified for LRRC15 (FIG. **6**A), suggesting that LRRC15 may require a more physiologically relevant setting for optimal activity. To test this hypothesis, LRRC15 was screened as gD-GPI (FIG. **7**A) and full-length (FIG. **7**B) receptor-rEVs using RDIMIS. Notably, both of these efforts identified similar sets of putative interactors for LRRC15, which were not described in available databases (FIG. **6**B).

[0538] CD248 was biotinylated using EZ-LINK™ Sulfo NHS-LC-LC-Biotin (Thermo Fisher) and cleaned up on a Zeba desalting column with a 7K MWCO (Thermo Fisher). CD248 was loaded onto Streptavidin (SA) Biosensors at 25 nM. LRRC15-Fc protein (Genentech) was provided at 500 nM. LRRC15 rEVs were provided at 0.25 mg/mL total protein (2.5-3.5 nM).

[0539] Since CD248 was a top scoring hits in both screens, its expression is upregulated in tumor stroma (Rouleau et al., *Clin. Cancer Res.,* 14: 7223-7236, 2008; Rouleau et al., *Int. J. Oncol.,* 39: 73-89, 2011; Teicher et al., 10: 993-100, 2019), and it has been suggested to promote tumor growth (Maia et al., *BMC Cancer,* 2: 1-12, 2011), the LRRC15-CD248 interaction was selected for further characterization. While LRRC15 and CD248 have been independently reported to be upregulated in solid tumors (Rouleau et al., *Clin. Cancer Res.,* 14: 7223-7236, 2008; Purcell et al., *Cancer Res.,* 78: 4059-4072, 2018), it was unclear whether they were expressed in the same tumor samples. Significant correlations between the expression of CD248 and LRRC15 (FIGS. **8**A, **8**B, **9**A, and **9**B) were identified in bulk RNA-seq data from The Cancer Genome Atlas (TCGA) for four different tumor indications. These correlations suggest that CD248 and LRRC15 are either found on the same cell type or are co-regulated. To distinguish between these two possibilities, single-cell RNA-seq data from head and neck cancer patients were re-analyzed to highlight LRRC15 and CD248 expression (Puram et al., *Cell,* 171: 1611-1624e.24, 2017). This analysis revealed that LRRC15 and CD248 are co-expressed on a subset of CAFs (co-occurrence score (Odds ratio)=9.44) (FIG. **8**C), with CD248 showing a broader expression that encompasses all CAF and cancer-associated pericyte (CAP) cells identified using markers such as DCN and RGS5 (FIG. **9**C).

[0540] Together, these results position RDIMIS as a robust method to identify new interactors for receptors not amenable to other technologies that rely on recombinant protein expression. Further, while it is unclear whether the interaction between LRRC15 and CD248 is occurring on the same cell or between cells, the above analysis suggests that these proteins have ample opportunity to interact in patient tumors, providing a potential biological context where this interaction might be relevant.

[0541] A. The LRRC15-CD248 Interaction Requires LRRC15 Expression on a Membrane

[0542] The LRRC15-CD248 interaction was further characterized using biophysical and biochemical methods. First, a miniature AVEXIS assay was performed using CD248 pentamerized ectodomains. Similar to LRRC15 (FIG. **6**A), no high-confidence hits were identified when CD248 pentamerized ectodomains were screened against the STM protein library (FIG. **10**A). Consistent with this result, no binding was observed between LRRC15 and CD248 recombinant proteins when the interaction was analyzed by either BLI or surface plasmon resonance, even when experimental conditions to maximize sensitivity of detection were employed (FIGS. **10**B and **11**A). BLI analysis confirmed lack of detectable binding when both LRRC15 and CD248 were tested as recombinant ectodomains. In contrast, the interaction was readily detectable when LRRC15 was displayed on EVs (FIG. **11**A). Similarly, this interaction could also be observed on the plasma membrane of a cell. First, rEVs displaying LRRC15 bound to CD248 overexpressed on the cell surface (FIG. **11**B) over ten times more than cells transfected with an empty vector control (FIG. **11**D). Second, LRRC15 was expressed on cells and incubated with CD248 recombinant protein that was biotinylated and tetramerized using fluorescently labeled streptavidin. Binding was readily detectable for LRRC15-expressing cells, but not for cells expressing a control protein (FIGS. **11**B-**11**D). These assays reinforce the notion that this interaction requires a membrane, but not specifically rEVs.

[0543] To better understand what might be underlying the membrane dependence of this interaction, the rEV membranes were altered either by disrupting them using Filipin III, which forms cholesterol ultrastructures (FIGS. **17**A-**17**C) or by depleting membrane cholesterol with methyl-β-cyclodextrin (MβCD) (FIGS. **17**D-**17**F) (Petro et al., *Toxicon,* 48: 1035-1045, 2006). That treating cells with 100 μM Filipin III or 15 mM MpβCD all but eliminated the binding of LRRC15 gD-GPI expressing vesicles to CD248 monomer as shown by BLI. These agents also affected the detection of the gD epitope tag by an anti-gD antibody. Interestingly, while 100 μM Filipin III also dramatically reduced the binding of LRRC15 FL rEVs to CD248, a minor effect was observed with MpβCD.

[0544] Though it was shown that LRRC15 and CD248 can interact in a membrane-dependent manner, it was not known whether they were present in the same physiological environment. Whereas LRRC15 and CD248 have been independently reported to be upregulated in solid tumors (Rouleau et al., *Clin. Cancer Res.* 14: 7223-7236, 2008; Purcell et al., *Cancer Res.,* 78: 4059-4072, 2018), it was unclear whether they were expressed in the same tumor samples. Bulk RNA-seq data from The Cancer Genome Atlas (TCGA) for four different tumor indications showed significant correlations between the expression of CD248 and LRRC15 (FIGS. **8**A, **8**B, and **9**A-**9**C). This correlation suggested that CD248 and LRRC15 are either found on the same cell type or that CD248 and LRRC15 are co-regulated. To help answer that question, single-cell RNA-seq data from head and neck cancer patients was re-analyzed to highlight LRRC15 and CD248 expression (Puram et al., *Cell,* 171: 1611-1624.e24, 2017). This revealed that LRRC15 and CD248 are co-expressed on a subset of CAFs (co-occurrence score (Odds ratio)=9.44) (FIG. **8**C), with CD248 showing a broader expression that encompasses all CAF and cancer-associated pericytes (CAPs) identified using markers such as DCN and RGS5 (FIG. **9**C). There are several models that may explain the membrane dependence of the interaction. The simplest model is that the LRRC15 ectodomain requires a membrane environment for proper folding. Interestingly, since both the full-length and gD-GPI-tagged ectodomain captured this interaction, the determinants responsible for this dependence may not be within the transmembrane domain or a precise spacing between the membrane and the ectodomain. Another plausible explanation is that the presence of the membrane promotes the formation of highly clustered arrays of receptors, increasing protein avidity beyond the tested pentamerization, to stabilize the interaction. This is in part supported by the evidence that Filipin III and MβCD can disrupt this interaction. In particular, Filipin III, which is thought to bind to but not remove hydroxysterols like cholesterol in the membrane (Bolard, *BBA-Rev. Biomembr.,* 864: 257-304, 1986), could be reducing the ability of receptors to cluster. While the Filipin III could be causing a general disruption to the membrane and therefore affecting both the full-length and gD-GPI tagged species, the effect of MpβCD was primarily on the gD-GPI tagged LRRC15. This is consistent with works suggesting that GPI-anchored receptors like the folate receptor depend on membrane cholesterol for clustering (Rothberg et al., *J. Cell Biol.,* 111: 2931-2938, 1990). Alternatively, the LRRC15-CD248 interaction may depend on the recruitment of a yet unknown factor that promotes or stabilizes the complex.

[0545] For immunofluorescence assays, HEK293T cells were split into 96-well SENSOPLATES™ (Greiner Bio-One) coated with 0.1 mg/mL Poly-D-Lysine (Gibco) for 30 minutes at 37° C. Cells were transfected using LTX Reagent (Thermo Fisher Scientific) according to the manufacturer's specifications. For fluorescent rEV experiments, rEVs were harvested from EXP1293F™ cells transiently co-transfected with gag-mNeonGreen and the receptor of interest. They were purified by ultracentrifugation at 100,000×g for 90 minutes after a 10 minute spin at 300×g and a 1 hr spin at 3,000×g to remove cells and debris. rEVs were resuspended in PBS and were incubated with cells for 30 minutes at 4° C. Cells were washed with PBS and fixed using 4% PFA for 10 minutes. CD248 protein (R&D Systems) was biotinylated using EZ-LINK™ Sulfo NHS-LC-LC-Biotin (Thermo Fisher Scientific), cleaned up on a Zeba 7K MWCO desalting column, and tetramerized using streptavidin-APC (Agilent). DNA was stained with 10 μg/mL Hoechst 33342 (Tocris Bioscience).

[0546] Altogether, these results confirm CD248 as a new interaction partner for LRRC15, and indicate that the LRRC15-CD248 interaction is facilitated in the context of a membrane. These findings highlight the advantages of RDI-MIS, which provides increased performance for detection of interactions that involve membrane proteins, and that are refractory to other technologies that necessitate the use of recombinant proteins.

[0547] Beyond identification of protein interactomes, the rEV-based receptor-display can provide quantitative explorations of receptor behavior in membranes. For example, BLI can be used to characterize the binding kinetics of a receptor with its ligand in a membrane (FIG. **11**A).

Example 6. G Protein-Coupled Receptor Interaction Screening Enables the Discovery of a Novel Receptor for PD-L1 (Programmed Cell Death Ligand 1) Called Adhesion GPCR B1 (ADGRB1)

[0548] A. Background

[0549] The G protein-coupled receptor (GPCR) superfamily encompasses nearly 20% of the extracellular proteins in the human genome and is the target of over one third of all FDA approved drugs. However, only two biological drugs, which normally disrupt extracellular protein interactions, target GPCRs. GPCRs have lagged behind in biological drug development partly because extracellular interaction mapping of GPCRs has lagged behind the rest of the human genome. Therefore, a GPCR-focused extracellular interaction screen was implemented to map the interactions of receptors targeted for immunotherapies in cancer. A novel receptor for PD-L1 (programmed cell death ligand 1) called adhesion GPCR B1 (ADGRB1) was observed. Secondly, the interactions of ADGRB1 were mapped against the majority of the single-transmembrane receptors in the human genome, and a novel interaction with ICOSLG (inducible T cell costimulatory ligand) was observed. These data demonstrate the potential for GPCR interaction screening in mapping new biology and developing new avenues for therapeutic interventions in cancer.

[0550] The field of interaction mapping, often referred to as "interactomics," was developed in order to understand cellular protein/protein interactions. Beginning with the development of the yeast-two-hybrid screen in 1989 (Young, Biology of Reproduction, 58(2): 302-311, 1998), interaction mapping has yielded many insights into how protein interaction networks function under physiological conditions, and in disease. Recently, the field of interactomics has exploded with thorough mapping of context-specific protein networks (Go et al., *Nature,* 595: 120-124, 2021; Huttlin et al., *Cell,* 184(11): 3022-3040, 2021). One of the major challenges in mapping protein/protein interactions is to include proteins embedded in cellular membranes. Defining interaction networks in the extracellular space is even more challenging, due to the typically transient interactions that often involve posttranslational modifications, such as cysteine reduction and glycosylation (Martinez-Martin, *J. Immunol. Res,* 2017: 2197615, 2017). Therefore, extracellular protein interaction mapping has lagged behind in the interactomics field, as new technologies have been required to drive the field forward.

[0551] One of the key insights into developing extracellular interaction technologies was the recognition that avidity is a critical component to deciphering transient, but physiologically relevant, extracellular protein interactions (Gonzalez, *Methods,* 57(4): 448-458, 2012). Screens have been developed to utilize avidity to map interactions across most of the extracellular proteins in the human genome. For example, the discovery that the poliovirus receptor (PVR) interacts with TIGIT (T cell immunoglobulin and ITIM domain) was made by screening potential proteins as Fc-tagged dimers (Yu et al., *Nature Immunology,* 10(1): 48-57, 2009). Microbeads have been used to multimerize ligands and screen against protein microarrays to map the extracellular interactome of the human adenovirus (Martinez-Martin et al., *Nature Communications,* 7: 11473, 2016). Similarly, baculovirus was used as a way to present receptors as a multimerized probe for screening against these protein microarrays (Tom et al., *Analytical Biochemistry,* 479: 1-5, 2015). Large-scale libraries of Fc-tagged extracellular proteins have been used to screen for interactions using microbeads (Husain et al., *Mol. Cell. Proteomics,* 18: 2310-2323, 2019) and also ligands that were multimerized by genetic fusion for avidity-based extracellular interaction screening (Martinez-Martin et al., *Cell,* 174(5): 1158-1171, 2018; Verschueren et al., *Cell,* 182: 329-344.e19, 2020). However, despite these successes, one major family of extracellular proteins has remained intractable in interactomic screens: multi-transmembrane receptors (MTMRs).

[0552] The human genome encodes more than 5,000 receptors and secreted proteins that interact with the extracellular space. Single-transmembrane receptors encompass over 2,000 of these extracellular proteins, while secreted proteins represent over 600 (Uhlén et al., *Science Signaling,* 12(609), 2019). The remaining less than 2,000 extracellular proteins are MTMRs and over 800 of those belong to the G protein-coupled receptor (GPCR) superfamily. The GPCR superfamily has a rich history of successful clinical therapeutics developed against it, with one third of all FDA approved drugs targeting just over 100 of these 800 GPCRs (Congreve et al., *Cell,* 181(1): 81-91, 2020). The remaining members of the GPCR superfamily include over 400 olfactory receptors, although these receptors have mostly restricted expression in the olfactory epithelium. However, olfactory receptors should not be excluded from consideration for drug development as they have been shown to be important drivers of physiology and dysregulated in disease models in tissues where the olfactory receptors are expressed outside of the olfactory epithelium (Pronin and Slepak, The *Journal of Biological Chemistry,* 296: 100475, 2021). The GPCR superfamily is vital in almost every physiological system and is such a successful drug target because the receptors are expressed at the plasma membrane but their expression is relatively low and restricted to specific cell types.

[0553] While the plasma membrane localization and low and restricted expression of the GPCR superfamily make these proteins ideal targets for drug development, these same properties present unique challenges for studying GPCR biology. Indeed, there are still over 100 orphan GPCRs with unknown ligands that control critical physiological functions (Laschet et al., *Biochemical Pharmacology,* 153: 62-74, 2018). Additionally, interaction mapping of GPCRs has lagged behind other extracellular proteins (Dunn et al., *Pharmacological Reviews,* 71(4): 503-519, 2019). GPCRs are also underrepresented in biological drug development. While GPCRs encompass over 30% of FDA-approved drugs, the GPCR superfamily is only the target of 2% of the FDA approved biological drugs (Hutchings, *Expert Opinion on Biological Therapy,* 20(8); 925-935, 2020. Biological drug development allows for robust protein interaction disruption or enhancement, tissue targeting, and conjugated drug delivery (Lu et al., *Journal of Biomedical Science,* 27(1): 1, 2020). Secondly, it is possible to develop complex pharmacophores to virtually any allosteric site that is available as an antigen; this potential could speed the development of next generation GPCR drug classes, such as biased agonists or allosteric modulators. Therefore, a set of GPCR-focused tools that would allow for interaction mapping and biological drug characterization were built.

[0554] The first GPCR-focused platform described herein is a cellular overexpression system that utilizes ligand multimerization to detect protein/protein interactions on the

cell surface. Secondly, GPCRs were packaged into recombinant extracellular vesicles (Geeurickx et al., *Nat. Commun.,* 10: 1-12, 2019) and screened for interactions against the recently published library of Fc-tagged single-transmembrane receptor extracellular domains (Verschueren et al., *Cell,* 182: 329-344.e19, 2020). Using these complementary approaches, orphan ligands can be screened across all of the GPCRs in the human genome and orphan GPCRs can be screened against most of the proteins in the extracellular space.

[0555] B. Employment of a Comprehensive Cell-Based Overexpression Interaction Screen Reveals Adhesion Receptor B1 (ADGRB1) is a Novel Receptor for PD-L1 (Programmed Cell Death Ligand 1)

[0556] A comprehensive library of multi-transmembrane receptors in mammalian overexpression vectors was developed in collaboration with the DNASU plasmid repository (Seiler et al., *Nucleic Acids Research,* 42 (Database issue), D1253-1260) to clone their collection of multi-transmembrane receptors in gateway donor vectors into the pT-Rex-DEST31 plasmid (Invitrogen). This created an N-terminal HIS tag that was detected on the surface of cells (FIG. **18**A). In addition, a G protein-coupled receptor-focused DNA library was created, with an N-terminal FLAG tag and C-terminal Venus. The GPCR collection allowed for the detection of low-expressing receptors (FIG. **18**B). In all, the HIS-tagged MTMR collection allows for mostly unlabeled receptors to be overexpressed at the cell surface (FIG. **18**C), while the GPCR-Venus collection allows for detection of low-expressing receptors above background staining (FIG. **18**D).

[0557] Using this comprehensive library, four fluorescently labelled peptide ligands were screened using high-throughput transfections and high-content imaging (FIG. **23**). EGF and RSPO3 were chosen because they have well-characterized receptors. PD-L1 and PVR bind to complex networks of the immunoglobulin superfamily of receptors and are the target of many biological therapeutics (Andrews et al., *Nat Immunol,* 20: 1425-1434, 2019). EGF-647 bound only to the control EGFR added to each plate as a transfection control (FIG. **19**A). R-spondin 3 (RSPO3) was fused to an Avidity AVITAG™ (Avi tag) to allow for biotinylation and tetramerization with an APC labeled streptavidin. This tetramerized RSPO3 bound to its known G protein-coupled receptors, leucine rich repeat GPCR (LGR) 4 and 5 (FIG. **19**B). The extracellular domain of the polio virus receptor (PVR) was tetramerized and found to only bind to the control single-transmembrane receptor CD226 that was added to the screen as a control (FIG. **19**C).

[0558] When the extracellular domain of programmed cell death ligand 1 (PD-L1) was screened as a fluorescent tetramer, it bound to controls and also to adhesion GPCR B1 (ADGRB1) (FIG. **19**D). Based on this unexpected finding, a method for mapping the complete interactions of one GPCR against families of potential interacting proteins was developed.

[0559] C. A Vesicle-Based Interaction Map for ADGRB1 and Leucine Rich GPCRs

[0560] Recombinant extracellular vesicles (rEVs) (Geeurickx et al., *Nat. Commun.,* 10: 1-12, 2019) were used in order to map the interactions of G protein-coupled receptors (GPCRs). rEVs are generated by co-transfecting a receptor of interest along with the GAG protein from HIV, as described in Example 1. GAG stimulates the production of microvesicles and exosomes (Geeurickx et al., *Nature* Protocols, 16: 603-633, 2021), which have a uniform packaging orientation (FIG. **20**A) and size (FIGS. **20**B-**20**D). GAG co-transfection enhanced the packaging of receptors into vesicles (FIGS. **20**E and **20**F), and GPCRs trafficked efficiently into rEVs (FIG. **20**G), as shown using biolayer interferometry (BLI). BLI allows for the detection of vesicles where the association curve is inverted (Cameron et al., Octet® Potency Assay: Development, Qualification and Validation Strategies. *Satorius Application Note,* 2021), allowing confident determination that the association is with an rEV-sized particle in our BLI readouts.

[0561] The poliovirus receptor (PVR) and programmed cell death ligand 1 (PD-L1) were packaged into vesicles, along with a fluorescently labelled GAG, and these rEVs were screened against the comprehensive multi-transmembrane library. As with the recombinant proteins shown in FIGS. **18**A-**18**D, PVR packaged into rEVs bound to only the control single-transmembrane receptors (FIG. **21**A) and PD-L1 bound to the control receptors as well as ADGRB1 (FIG. **21**B).

[0562] Next, the recently published (Martinez-Martin et al., *Nature Communications,* 7: 11473, 2016; Verschueren et al., *Cell,* 182: 329-344.e19, 2020) avidity-based extracellular interaction library of proteins was adapted to map the interactions of GPCRs packaged into rEVs. Briefly, the library comprises a large collection of Fc-tagged proteins in mammalian expression vectors with a signal sequence for secretion into the media. After transfection, the conditioned media is incubated on Protein A coated white plates, thus capturing the proteins from the media, as described above. For this screen, each GPCR was fused to Rluc8 (Loening et al., *Protein Engineering, Design & Selection,* 19(9): 391-400, 2006) in order to allow for rapid and sensitive detection of receptor-ligand interactions. GPCRs packaged into rEVs binding to a member of the protein library were detected using luciferase-produced light.

[0563] ADGRB1 was screened against the collection of single-transmembrane receptor extracellular domains (STM library) fused to Fc (FIG. **21**C). The interactions between ADGRB1 and RTN4R family members (Chong et al., *Genome Biology,* 19(1): 205, 2018), as well as PD-L1, were confirmed. A number of novel interactions were also uncovered, including ICOSLG, a protein related to PD-L1 (Table 15; Greenwald et al., *Annual Review of Immunology,* 23: 515-548, 2005). FIGS. **26**A-**26**F show the results of screens for binding of EVs comprising ADGRB1, LGR4, or LGR5 to the STM library or to a library of secreted proteins fused to Fc. Novel interactions identified in these screens are shown in Table 15.

TABLE 15

Interactions identified in screen	
ADGRB1	PD-L1
	ICOSLG
	DNER
	CNTN6
LGR4	CLPS
	EDIL3
	IZUMO4
	IZUMO1
	BTNL3
	CD93
	CEACAM16

TABLE 15-continued

Interactions identified in screen	
LGR5	IL-6 LRRC4C SCARF1 TRIL CLPS EDIL3 IZUMO4 CD93 GPR125 IL6R SCARF1 TRIL

[0564] D. Recombinant Proteins Confirm ADGRB1 Binders Found

[0565] Recombinant proteins were used to confirm some the interactions (FIG. **22**A). Cells overexpressing ADGRB1 fused to Venus (FIG. **22**B) were treated with recombinant PD-L1, ICOSLG, or RTN4R fused to an Fc tag and robust binding was observed by staining for the Fc tag. This binding was not observed for ADGRB2 or ADGRB3 (FIGS. **24**A-**24**C).

[0566] E. Discussion

[0567] G protein-coupled receptor (GPCR) interaction mapping is a powerful, yet underdeveloped area of research. In this Example, two interaction-mapping platforms have been adapted to accommodate GPCRs (FIG. **23**). The first is a cell-based platform that can be used to uncover novel receptors for orphan ligands. Here, this cell-based platform was used to discover a previously unappreciated receptor for PD-L1 called adhesion GPCR B1 (ADGRB1). Secondly, an avidity-based interaction screen was implemented using GPCRs in recombinant extracellular vesicles (rEVs), as described above. In this way, a GPCR of interest can be screened against libraries of potential interacting partners. Using this vesicle-based platform, ADGRB1 was shown to bind to ICOSLG.

[0568] ADGRB1 belongs to a family of 33 adhesion GPCRs that share a remarkable long N-terminus. Adhesion GPCRs are thought to autoproteolyze this long extracellular domain in the Golgi apparatus but remain complexed together at the plasma membrane. Upon binding of ligands, the large extracellular domain dissociates from the seven transmembrane domain, and the remaining short stalk activates the seven transmembrane domain, in a similar way to the protease activated receptor family (Nijmeijer et al., *Biochemical Pharmacology,* 114: 88-102, 2016). Adhesion GPCRs have been shown to activate G proteins and also recruit arrestins (Kishore et al., The *Journal of Biological Chemistry,* 291(7): 3385-3394). The large extracellular domain of ADGRB1 binds to lipopolysaccharides and phosphatidylserine, and receptor activation is believed to drive engulfment of bacterial cells and apoptotic cells (Park et al., *Nature,* 450(7168): 430-434, 2007; Das et al., *The FASEB Journal,* 28(5): 2214-2224). ADGRB1 expression on macrophages was previously demonstrated (Park et al., *Nature,* 450(7168): 430-434, 2007); however, that observation has recently been challenged (Hsiao et al., *Frontiers in Immunology,* 10: 962, 2019). ADGRB1 is also known to be a tumor suppressor gene that is down-regulated in cancer cells (Zhu et al., *Cancer Cell,* 33(6): 1004-1016e15, 2018).

[0569] It is interesting to note that ADGRB1 binding to two of the classical ligands for T cell activation (PD-L1 and ICOSLG) was observed. However, these ligands are known to have opposing effects on T cell activation, where PD-L1 inhibits T cells and ICOSLG activates them (Greenwald et al., *Annual Review of Immunology,* 23: 515-548, 2005). One of the ways that tumors evade T cells is by overexpressing PD-L1, which drives the silencing of the T cells, thus allowing tumors to evade the immune system. Indeed, PD-1/PD-L1 has been targeted by biological drugs to disinhibit T cell silencing and is a proven clinically effective immunotherapy for cancer (Lee et al., *Scientific Reports,* 7(1): 5532, 2017). The interaction of ADGRB1 with PD-L1 and ICOSLG may offer a new avenue for drug development, as PD-1/PD-L1 blockade can be ineffective (Lee et al., *Frontiers in Pharmacology,* 12: 681320, 2021).

[0570] One unanswered question is the impact of the interaction of ADGRB1 with PD-L1 and ICOSLG on signaling. It has been difficult to develop an ADGRB1-based signaling model in heterologous cell lines (FIGS. **25**A-**25**D). Recently, another adhesion GPCR family member was shown to activate $G_{\alpha i}$ in response to N-termini binding and also by treatment with cholesterols, which bind directly to the seven transmembrane domain (Ping et al., *Nature,* 589 (7843), 620-626, 2021). Previous work has shown that ADGRB1 is not cleaved in HEK cells (Araç et al., *The EMBO Journal,* 31(6): 1364-1378, 2012), and also that ADGRB1 signals through a non-canonical effector called ELMO (Park et al., *Nature,* 450(7168): 430-434, 2007). Activation of ADGRB1 may be only possible in relevant cell lines or in vivo.

[0571] GPCRs are a critical target for many drug development programs. Many small molecule agents against GPCRs have successfully been brought to the clinic. However, novel drug modalities, such as biologicals, have lagged behind small molecules for GPCR drug development. Novel modalities, especially antibody-based biologicals, offer a robust way to target extracellular protein interactions and also to modulate receptor activation. Here, methods for mapping GPCR extracellular interactions were developed and new interactions that have implications for cancer immunotherapies were found.

[0572] F. Materials and Methods

[0573] Cell Culture

[0574] HEK 293T and COS7 cells were maintained in DMEM+10% FBS, 10 mM HEPES pH 7.4, and Penicillin-Streptomycin (100 U/mL). EXPI293F™ cells were cultured in EXPI293™ expression media shaking at 150 RPM.

[0575] Multi-Transmembrane Receptor Library Generation

[0576] Receptors were cloned into either the pT-Rex-DEST31 plasmid (Invitrogen) or the pRK plasmid (Genentech) and sequence verified. 100 ng of receptor DNA (at 10 ng/μL for 10 μL/well) was plated into each well of a 384 well black Aurora Microplate (cat #ABC2-312-1B-PDL). DNA-printed Aurora plates were sealed and stored at −20° C. until the day of the experiment.

[0577] On the day of the transfection, DNA-printed Aurora plates were thawed at room temperature and spun down. 20 μL of Opti-MEM™ (Thermo Fisher, cat #11058021) with LIPOFECTAMINE™ LTX diluted 1:0.0072 and PLUS™ reagent diluted 1:0.0024 (Thermo Fisher, cat #15338100) was added to each well of the 384 well plate and incubated for 20 minutes at 37° C. with 5% CO_2. Afterwards, 20 μL of COS7 cells, diluted at 150,000 cells/

mL in DMEM+10% FBS, HEPES and P/S, were added to each well and incubated at 37° C. with 5% CO_2 for 48 hours.

[0578] Receptor Expression Analysis

[0579] After 48 hours, expression was verified by receptor fluorescence. Cells were starved in Opti-MEM™+5% BSA for 45 minutes at 37° C. with 5% CO_2. Next, rabbit anti-HIS antibody (Cell Signaling, cat #2365) or mouse anti-FLAG antibody (Sigma, cat #F3165) were diluted 1:1000 in Opti-MEM™+5% BSA and incubated on cells for 45 minutes at 4° C. Cells were then washed in PBS+Ca/Mg and fixed in 4% PFA for 20 minutes at room temperature, washed again in PBS+Ca/Mg, stained with a rabbit or mouse Alexa-647 secondary antibody diluted 1:1000 in Opti-MEM™+5% BSA at room temperature. The cells were then washed, then DAPI stained (Thermo Fisher, cat #62248) at 1 μg/mL for 20 minutes at room temperature, washed again. and stored in PBS+Ca/Mg at 4° C. until the day of imaging.

[0580] Receptor expression was evaluated by capturing two imaging fields using a 10× objective on an IN Cell Analyzer 6000 (GE Healthcare) with preset filters for DAPI, GFP and Cy5. The IN Cell Analyzer 6000 Development software was used to draw regions of interest for the DAPI, GFP, and Cy5 channels and count the total number of objects as well as the pixel density for the entire field. Total expression is expressed as the GFP channel divided by the total number of cells (DAPI counts). Surface expression is expressed as the antibody channel (Cy5) divided by total number of cells.

[0581] Cell-Based Interaction Screening

[0582] Similar to the receptor expression analysis, cells were blocked 48 hours after transfection in Opti-MEM™+ 5% BSA. Instead of primary antibodies, cells were treated with 100 nM tetramerized ligand diluted in Opti-MEM™+ 5% BSA for 45 minutes at 4° C. Tetramers were prepared as previously described (Verschueren et al., *Cell,* 182(2): 329-344.e19, 2020) with minor modifications. Briefly, the total mass of streptavidin-APC (Agilent, PJ27S) and the biotinylated protein of interest (RSPO3 and PVR were produced in-house, PD-L1 was purchased from Bio-Techne, cat #AVI156) was calculated. The total volume of protein needed was aliquoted and the volume of streptavidin was added in four steps with 10-minute incubations at room temperature in between. After the final addition and incubation, the tetramer was diluted to a final concentration of 100 nM in Opti-MEM™+5% BSA. For EGF-647, the fluorescent protein was purchased from Thermo Fisher (cat #E35351) and diluted to 100 nM in Opti-MEM™+5% BSA. Cells were then washed with PBS+Ca/Mg and fixed in 4% PFA for 20 minutes at room temperature, washed again, and DAPI stained (Thermo Fisher, cat #62248) and stored at 4° C. in PBS+Ca/Mg until the day of imaging.

[0583] For the vesicle-based interaction screening, vesicles were added instead of tetramerized ligand, and GAG was fused to Neon Green.

[0584] Imaging was done as described above for the receptor expression analysis.

[0585] Recombinant Extracellular Vesicle Preparations

[0586] 100 mL of EXPI293F™ cells were transfected with 100 μg of DNA, with a split of 50 μg of GAG DNA and 50 μg of receptor. Seven days after transfection, the cells were spun down and media was filtered through a 0.2 micron filter. Protease inhibitor (Roche) was added to the filtered media and it was spun at a slow speed of 2,000×g for 30 minutes. Next, the media was spun at 100,000×g for 90 minutes. Vesicle pellets were reconstituted in PBS+Ca/Mg and stored until the day of experiments.

[0587] Electron Microscopy

[0588] Electron microscopy was performed as described herein. The suspension of vesicles was adsorbed for 15 minutes to the surface of formvar and carbon coated TEM grids. After a short rinse with distilled water, the sample was stained with 2% phosphotungstic acid (PTA) for EV prep cleanup for 60 seconds and then air dried. Samples were then washed with PBS for 15 minutes, with water for 1 minute, and stained with 1% uranyl acetate for 1 minute before being blotted and air dried. Imaging was done with a JEOL JEM-1400 transmission electron microscope (TEM) and a GATAN ULTRASCAN® 1000 CCD camera at magnifications from 5000× to 50000×. Scale bars are indicated in the images.

NanoSight

[0589] Vesicles were diluted in clean PBS and NanoSight (Malvern Panalytical) data was collected and averaged for three runs with the clean PBS used as a baseline.

[0590] Biolayer Interferometry

[0591] Vesicles were diluted into PBS and the gD antibody (abcam, cat #ab6507) and FLAG antibody (Sigma, cat #F3165) were diluted 1:10 in PBS; TIGIT-Fc (Bio-techne cat #7898-TGB) was diluted to 100 μg/mL. AMC tips (Sartorius, cat #18-5088) or AHC tips (cat #18-5060) were used to capture the antibodies or Fc tag, respectively.

[0592] Vesicle-Based Interaction Screening

[0593] Vesicle-based interaction screens were performed as described above. For the control proteins and follow-up experiments, recombinant proteins and antibodies were added to empty wells of the white 384 well protein A coated plates (Thermo Fisher, cat #NCI15133) and allowed to incubate for at least 24 hours at 4° C.

[0594] Ligand Binding

[0595] For ligand binding, the same protocol was used as for the interaction screening, except that receptors of interest were transiently overexpressed in HEK cells using calcium phosphate transfections, then plated onto Aurora imaging plates. Instead of tetramerized ligand, Fc-fused proteins (BIO-TECHNE®, PD-L1 cat #AVI156, ICOSLG cat #AVI165, and RTN4R cat #1208-NG) were incubated on cells at a concentration of 10 μg/mL (~70 nM for each dimer). Anti-human Fc fused to Alexa-647 was then used as a secondary antibody, and images were captured on an IN Cell Analyzer 6000.

Example 7. Cell-State Profiling Using rEV-Based RDIMIS in Different Cell Lines

[0596] The above Examples demonstrate that rEVs can be used to identify and characterize interactions with receptors of interest. In addition, a number of binding partners that interact with rEVs in general were identified (see, e.g., Example 4). These interactions may capture a snapshot of the state of the cells of origin (parent cells) for the rEVs, as receptors of interest and many other membrane and cytoplasmic proteins appear to be incorporated into rEVs at levels proportional to their expression levels in the parent cells.

[0597] It is therefore hypothesized that rEVs can capture cell surface proteins (e.g., can capture a representative sample of the cell surface proteins at a given time point) and

RDIMIS can thus be used to profile interactions that are relevant for cells with different cell states. Specifically, rEVs may be used as a proxy for cellular interactions with the rEVs' parent cells and may capture important interactions that arise due to, e.g., disease-specific stimulation, differentiation, and cell-type differences. One advantage of this approach is that it captures not only the aggregate binding behavior of cell-surface proteins, including complexes and complicated interaction dynamics, but also interactions that depend on non-protein components like glycosylation marks and lipids.

[0598] Methods

[0599] Stable cell lines that express a vesicle budding factor and a readout for RDIMIS (e.g., a vesicle budding factor attached to a readout for RDIMIS, e.g., gag-*Renilla* luciferase (gag-RLuc)) are generated for cell lines including but not limited to: immune cell lines representing T-cells (JURKATs), B-cells (BJABs) and monocytes (THP1s); neuronal cell lines; and fibroblast cell lines. rEVs are generated from these cell lines as described in Example 1.

[0600] The cell lines and rEVs generated therefrom are implemented for:

[0601] 1. Characterization of differences in the interaction profiles of rEVs from different cell lines representative of different cell types; and

[0602] 2. Characterization of the interaction profiles of rEVs from the same cell line over time (e.g., at time points before and after the addition of stimuli, before and after inducing signaling, and/or before and after induction of a disease-related state (e.g., immune exhaustion) or at two or more time points in a differentiation pathway or profile). In one approach, rEVs are collected at select time points, e.g., after stimuli are applied. In another approach, the cell line is modified such that the budding factor is present (e.g., expressed) only during selected periods. Expression of the budding factor may be controlled using:

[0603] (a) an inducible promoter that relieves or suppresses the expression of the budding factor after the addition of a small molecule (e.g., a cell-permeable small molecule), e.g., the T-REX™ System;

[0604] (b) a small molecule-induced degradation system in which a protein (e.g., the budding factor) is rapidly degraded upon induction (e.g., the TIR1 auxin inducible degron (AID) system); or

[0605] (c) a small molecule-induced stabilization system in which a protein (e.g., the budding factor) comprises a degradation domain and the protein is protected from degradation upon induction (e.g., the Shield-1-FKBP system).

[0606] Integration of constructs can be performed, e.g., by using CRISPR-Cas9 or genome engineering techniques to insert the construct into a safe-harbor locus. Alternatively, methods of random integration such as the PiggyBac Transposon System (SBI) may be used.

Example 8. Use of EVs to Measure Membrane Protein-Associated Enzymatic Activities

[0607] The above Examples demonstrate that EVs can be used to display membrane proteins and membrane-associated proteins in the context of a membrane. This includes proteins with enzymatic activity, such as peptidases, proteases, and phosphatases. The activities of such proteins can be detected and assayed on EVs, thus allowing biochemical characterization of the enzymatic effects of membrane proteins in or on membranes using assays usually designed for recombinant proteins.

[0608] In one approach, rEVs comprising proteins with enzymatic activity are used in enzymatic assays for membrane proteins. In another approach, rEVs comprising proteins with enzymatic activity are used in drug discovery for, e.g., molecular glues, wherein peptidases, proteases, phosphatases, and kinases can be recruited to another membrane protein on the same EV or to inhibit specific membrane-bound enzymatic activities.

[0609] Peptidase Activity Assay

[0610] As a proof of concept, it was tested whether the peptidase activity of Carboxypeptidase M (CPM) could be detected and assayed on vesicles. Either full-length (FL) or gD-GPI (gD) CPM was expressed in vesicles.

[0611] An assay for peptidase activity was performed as follows. This assay was modified from the manufacturer's provided protocol for use of Recombinant Human Carboxypeptidase M Protein (R&D SYSTEMS®).

[0612] 1. EVs were diluted. 200 μL Assay Buffer was added, and further reagents were added as listed below.

[0613] 2. The substrate Bz-Ala-Arg-OH (50 mM stock solution) was diluted to 1 mM in Assay Buffer (126 μL in 6300 μL Assay Buffer)

[0614] 3. 150 μL of EVs and 150 μL 1 mM substrate were mixed. Controls containing 150 μL of 1 mM substrate only were prepared.

[0615] 4. Reactions were incubated for 10 minutes at room temperature.

[0616] 5. Reactions were stopped by adding 300 μL of a solution containing 15 mM o-PA in 0.2 M NaOH containing 0.1% (v/v) 2-Mercaptoethanol and mixing well. (1260 μL 2M NaOH+12.6 μL Bme+507 μL o-PA+10.8 mL water)

[0617] 6. 150 μL of EVs was added to controls after stopping the reaction.

[0618] 7. All samples were incubated for 10 minutes at room temperature.

[0619] 8. 195 μL of the incubated samples were loaded in triplicate into a plate.

[0620] 9. Remaining EV solution was also added to plates too to confirm no substrate background.

[0621] 10. Samples were read at excitation and emission wavelengths of 330 nm and 450 nm (top read), respectively, in endpoint mode.

[0622] Results of the assay are shown in FIG. **27**. Robust peptidase activity on CPM-expressing EVs was detected (FIG. **27**). This activity was significantly greater than that observed in EVs not overexpressing CPM (pRK EV; vector control showing background levels of peptidase activity). This activity was more readily detectable compared to peptidase activity observed using recombinant protein.

[0623] Kinase Activity Assay

[0624] It was also demonstrated that signs of kinase activity can be detected on vesicles: as shown in FIG. **28**, co-expression of EPHA3 EVs with the EPHA3 ligands EFNA1-Fc and EFNA5-Fc enhanced the amount of EPHA3 phosphorylated species detected in vesicles. EVs were lysed and put into sample buffer and run on an acrylamide gradient gel to separate the protein species. They were then blotted with EPHA3, phospho-specifics, tubulin antibodies, and primary antibodies and detected with either fluorescently tagged secondary antibodies (680 and 800 nm LI-COR®

dyes) or anti-human antibodies to detect the Fc region on the various proteins being expressed. The blot was then imaged on a LI-COR® instrument. The same samples were loaded separately onto 2 different gels corresponding to the top and bottom images.

Example 9. Use of Generic EV Binders for Vesicle Purification

[0625] The gold standard for EV purification to date is ultracentrifugation, which is a long and cumbersome process. While there are some affinity-based methods to purify specific EVs, they are either too dirty, receptor-specific, or are designed for too small a scale to be useful for RDIMIS screening and other applications that require large quantities of EVs.

[0626] As described in Example 4, generic vesicle binders have been identified. These generic vesicle binders can be used to affinity purify EVs directly from conditioned medium. The generic vesicle binders have been ranked by ability to bind to the pure recombinant EVs described herein (Table 9), and they can be used to purify human EVs in general. In one approach, the binding conditions described in the above examples are reproduced on a column. In one approach, the column comprises Protein A-functionalized beads (e.g., is a PROTEIN A SEPHAROSE® column). Conditioned media containing one or more of the top generic EV binders (Table 9) modified to comprise an Fc region (e.g., a human Fc region) is flowed over the column, the column is washed, and then conditioned media containing EVs is flowed over the column. The EVs are then eluted using appropriate methods, e.g., harsh washes like high salt and/or specific ligands like sialoglycans for the SIGLEC family of proteins. A quick ultracentrifugation-based cleanup may be performed if necessary.

membrane-associated protein is expressed at or above a threshold level on the surface of the BV; and

(c) detecting an interaction between the heterologous membrane-associated protein and the at least one target polypeptide, thereby identifying a protein-protein interaction.

2. The method of claim **1**, wherein one or more of the target polypeptides is immobilized to a distinct location on the one or more solid surfaces, and wherein detecting an interaction comprises detecting a signal at a location on the solid surface that is above a threshold level.

3. (canceled)

4. The method of claim **1**, wherein the membrane-budding agent is selected from the group consisting of a HIV gag protein, Acyl.Hrs, ARRDC1, and ARF6.

5-10. (canceled)

11. The method of claim **1**, wherein the interaction is a transient interaction or a low-affinity interaction.

12. (canceled)

13. The method of claim **1**, wherein:

(i) the heterologous membrane-associated protein is a full-length protein; or

(ii) the heterologous membrane-associated protein comprises a protein fragment, a tag, and an anchor.

14-15. (canceled)

16. The method of claim **13**, wherein the anchor is a glycosylphosphatidyl-inositol (GPI) polypeptide and/or the tag is a glycoprotein D (gD) polypeptide.

17-31. (canceled)

32. A BV comprising (a) a heterologous membrane-associated protein comprising a protein fragment, a tag, and an anchor, wherein the heterologous membrane-associated protein is present on the outer face of the BV and (b) a membrane-budding agent.

SEQUENCE LISTING

```
Sequence total quantity: 1
SEQ ID NO: 1          moltype = AA  length = 500
FEATURE               Location/Qualifiers
source                1..500
                      mol_type = protein
                      organism = Human immunodeficiency virus
SEQUENCE: 1
MGARASVLSG GELDRWEKIR LRPGGKKKYK LKHIVWASRE LERFAVNPGL LETSEGCRQI  60
LGQLQPSLQT GSEELRSLYN TVATLYCVHQ RIEIKDTKEA LDKIEEEQNK SKKKAQQAAA  120
DTGHSNQVSQ NYPIVQNIQG QMVHQAISPR TLNAWVKVVE EKAFSPEVIP MFSALSEGAT  180
PQDLNTMLNT VGGHQAAMQM LKETINEEAA EWDRVHPVHA GPIAPGQMRE PRGSDIAGTT  240
STLQEQIGWM THNPPIPVGE IYKRWIILGL NKIVRMYSPT SILDIRQGPK EPFRDYVDRF  300
YKTLRAEQAS QEVKNWMTET LLVQNANPDC KTILKALGPG ATLEEMMTAC QGVGGPGHKA  360
RVLAEAMSQV TNPATIMIQK GNFRNQRKTV KCFNCGKEGH IAKNCRAPRK KGCWKCGKEG  420
HQMKDCTERQ ANFLGKIWPS HKGRPGNFLQ SRPEPTAPPE ESFRFGEETT TPSQKQEPID  480
KELYPLASLR SLFGSDPSSQ                                              500
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1. A method for identifying a protein-protein interaction, the method comprising:

(a) providing a collection of target polypeptides that are immobilized on one or more solid surfaces;

(b) contacting the collection of step (a) with a biological vesicle (BV) comprising a heterologous membrane-associated protein and a membrane-budding agent under conditions permitting the binding of the heterologous membrane-associated protein and at least one of the target polypeptides, wherein the heterologous

33. The BV of claim **32**, wherein the BV is produced by a process comprising (i) providing a parent cell that has been modified to express the heterologous membrane-associated protein and the membrane-budding agent and (ii) isolating the BV from the parent cell.

34. The BV of claim **32**, wherein the membrane-budding agent is selected from the group consisting of a HIV gag protein, Acyl.Hrs, ARRDC1, and ARF6.

35. (canceled)

36. The BV of claim **32**, wherein the anchor is a GPI polypeptide and/or the tag is a gD polypeptide.

37-55. (canceled)

56. A method of identifying a modulator of the interaction between a protein of Table 1 and a protein of Table 2, the method comprising:

(a) providing a candidate modulator;

(b) contacting a protein of Table 1 with a protein of Table 2 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 1 to the protein of Table 2, wherein the protein of Table 1 and the protein of Table 2 are reported to interact in Table 3; and

(c) measuring the binding of the protein of Table 1 to the protein of Table 2, wherein an increase or decrease in binding in the presence of the candidate modulator relative to binding in the absence of the candidate modulator identifies the candidate modulator as a modulator of the interaction between the protein of Table 1 and the protein of Table 2.

57-81. (canceled)

82. A method for identifying a biological vesicle (BV) having an altered binding profile, the method comprising:

(a) providing a collection of target polypeptides that are immobilized on one or more solid surfaces;

(b) contacting the collection of step (a) with a BV of interest;

(c) detecting an interaction between the BV of interest and the at least one target polypeptide, thereby identifying an interaction profile; and

(d) comparing the interaction profile of the BV of interest to the interaction profile of a control BV, wherein a difference between the interaction profile of the BV of interest and the interaction profile of the control BV identifies the BV of interest as one having an altered binding profile.

83-88. (canceled)

89. The method of claim **82**, wherein the BV of interest is derived from a sample from a subject.

90. The method of claim **89**, wherein the BV of interest and the control BV are derived from different tissues or different cell types.

91. The method of claim **89**, wherein the BV of interest is derived from a diseased tissue and the control BV is derived from healthy tissue.

92. A method of identifying a modulator of the interaction between a protein of Table 5 and a protein of Table 6, the method comprising:

(a) providing a candidate modulator;

(b) contacting a protein of Table 5 with a protein of Table 6 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 5 to the protein of Table 6, wherein the protein of Table 5 and the protein of Table 6 are reported to interact in Table 7; and

(c) measuring the binding of the protein of Table 5 to the protein of Table 6, wherein an increase or decrease in binding in the presence of the candidate modulator relative to binding in the absence of the candidate modulator identifies the candidate modulator as a modulator of the interaction between the protein of Table 5 and the protein of Table 6.

93-130. (canceled)

131. A method for characterizing an interaction profile of a cell line that has been modified to comprise a membrane-budding agent, the method comprising characterizing an interaction profile of a BV produced by the cell line.

132. (canceled)

133. A method for identifying a change in the interaction profile of a cell line that has been modified to comprise a membrane-budding agent, the method comprising:

(a) characterizing an interaction profile of a BV produced by the cell line at a first time point;

(b) characterizing an interaction profile of a BV produced by the cell line at a second time point; and

(c) comparing the interaction profile of the BV produced at the first time point to that of the BV produced at the second time point, wherein a difference between the interaction profile of the BV produced at the first time point and that of the BV produced at the second time point identifies a change in the interaction profile of the cell line.

134-136. (canceled)

137. The method of claim **133**, wherein the method comprises exposing the cell line to a stimulus following the first time point and before the second time point.

138-143. (canceled)

144. A method for identifying a difference in the interaction profiles of two cell lines that have been modified to comprise a membrane-budding agent, the method comprising:

(a) characterizing an interaction profile of a BV produced by the first cell line;

(b) characterizing an interaction profile of a BV produced by the second cell line; and

(c) comparing the interaction profile of the BV produced at the first cell line to that of the BV produced by the second cell line, wherein a difference between the interaction profile of the BV produced by the first cell line and that of the BV produced by the second cell line identifies a difference in the surface protein profiles of two cell lines.

145-155. (canceled)

156. The method of claim **1**, wherein the BV is produced by a process comprising (i) providing a parent cell line that has been modified to express the membrane-budding agent under inducible control; (ii) inducing expression of the membrane-budding agent, and (iii) isolating the BV from the parent cell line.

157-175. (canceled)

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