BIOLOGICAL VESICLES DISPLAYING CELL SURFACE PROTEINS AND METHODS RELATED TO SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Patent Application No. 63/120,167, filed on December 1, 2020; U.S. Patent Application No. 63/212,021, filed on June 17, 2021; and U.S. Patent Application No. 63/227,039, filed on July 29, 2021, the entire contents of each of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 29, 2021, is named 50474-231WO4_Sequence_Listing_11_29_21_ST25 and is 4,713 bytes in size.

FIELD OF THE INVENTION

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

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.

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

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.

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

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

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

In some examples, 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 examples, the detectable marker is an enzyme that produces a fluorescent signal in the presence of a substrate. In some examples, the enzyme is Renilla luciferase (Rluc) and the substrate is Rluc substrate.

In some examples, 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 examples, the membrane marker is a cholesterol marker. In some examples, the cholesterol marker is AMPLEX™ Red.

In some examples, the interaction is a transient interaction.

In some examples, the interaction is a low-affinity interaction.

In some examples, the heterologous membrane-associated protein is a full-length protein.

In some examples, the heterologous membrane-associated protein comprises a protein fragment, a tag, and an anchor.

In some examples, the anchor tethers the protein fragment to the surface of a membrane of a BV. In some examples, the anchor is a glycosylphosphatidyl-inositol (GPI) polypeptide.

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

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

In some examples, 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.

In some examples, the tag comprises a fluorescent protein.

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

In some examples, the protein fragment is an extracellular domain.

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

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

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

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.

In another example, 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.

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

In some examples, the anchor tethers the protein fragment to the surface of a lipid membrane of a BV. In some examples, the anchor is a GPI polypeptide.

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

In some examples, the tag comprises a fluorescent protein.

In some examples, the protein fragment is an extracellular domain of a transmembrane receptor. In some examples, the transmembrane receptor is a STM receptor.

In some examples, 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.

In some examples, 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.

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

In some examples, the BV comprises a membrane marker. In some examples, the membrane marker is a cholesterol marker. In some examples, the cholesterol marker is AMPLEX™ Red.

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

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

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.

In another example, 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.

In another example, 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.

In some examples, 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).

In some examples, the modulator is an inhibitor of the downstream activity of the protein of Table 1 or Table 2. In some examples, the modulator is an activator of the downstream activity of the protein of Table 1 or Table 2.

In some examples, the change in the downstream activity is a decrease in the amount, strength, or duration of the downstream activity. In some examples, the change in the downstream activity is an increase in the amount, strength, or duration of the downstream activity.

In some examples, 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).

In some examples, 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 examples, the antibody or antigen-binding fragment thereof binds the protein of Table 1. In some examples, the antibody or antigen-binding fragment thereof binds the protein of Table 2.

In some examples, the protein of Table 1 is LRRC15. In some examples, the protein of Table 2 is TEM1. In some examples, the downstream activity is tumor growth. In some examples, tumor growth is

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

In some examples, the modulator is an antibody or antigen-binding fragment thereof targeting LRRC15.

In some examples, the modulator is an antibody or antigen-binding fragment thereof targeting TEM1.

In another example, 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.

In another example, 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.

In another example, 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 examples, the increase or decrease in binding is at least 70%, as measured by an SPR assay, a BLI assay, or ELISA.

In some examples, the downstream activity is tumor growth.

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

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.

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

In some examples, the BV of interest is an engineered BV.

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

In another example, 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.

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.

In another example, 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.

In another example, 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.

In some examples, 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).

In some examples, the modulator is an inhibitor of the downstream activity of the protein of Table 5 or Table 6.

In some examples, the modulator is an activator of the downstream activity of the protein of Table 5 or Table 6.

In some examples, the change in the downstream activity is a decrease in the amount, strength, or duration of the downstream activity. In some examples, the change in the downstream activity is an increase in the amount, strength, or duration of the downstream activity.

In some examples, 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).

In some examples, 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 examples, the antibody or antigen-binding fragment thereof binds the protein of Table 5.

In some examples, the antibody or antigen-binding fragment thereof binds the protein of Table 6.

In some examples, the protein of Table 5 is ADGRB1.

In some examples, the protein of Table 6 is PD-L1.

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

In some examples, the modulator is an antibody or antigen-binding fragment thereof targeting PD-L1.

In some examples, the protein of Table 6 is ICOSLG.

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

In some examples, the modulator is an antibody or antigen-binding fragment thereof targeting ICOSLG.

In some examples, the modulator is an antibody or antigen-binding fragment thereof targeting ADGRB1.

In another example, 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.

In another example, 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-L1.

In another example, 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 examples, the increase or decrease in binding is at least 70%, as measured by an SPR assay, a BLI assay, or ELISA.

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

In another example, 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.

In another example, 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.

In another example, 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 examples, the increase or decrease in binding is at least 70%, as measured by an SPR assay, a BLI assay, or ELISA.

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

In another example, 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.

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.

In another example, 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.

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.

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

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

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

In some examples, the stimulus is a condition or agent that induces differentiation.

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

In another example, 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.

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.

In some examples, expression of the membrane-budding agent is inducible.

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

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

In some examples, 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.

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

In some examples, the method further comprises characterizing a cytoplasmic protein profile of the BV.

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

In another example, 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 examples, the membrane-budding agent is selected from the group consisting of a HIV gag protein, Acyl.Hrs, ARRDC1, and ARF6.

In some examples, the parent cell line is a mammalian cell line.

In some examples, the BV is an extracellular vesicle (EV).

In another example, 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.

In some examples, the membrane-associated protein is a peptidase and the assay for enzymatic activity is an assay for peptidase activity.

In some examples, the membrane-associated protein is a protease and the assay for enzymatic activity is an assay for protease activity.

In some examples, the membrane-associated protein is a kinase and the assay for enzymatic activity is an assay for kinase activity.

In some examples, the membrane-associated protein is a phosphatase and the assay for enzymatic activity is an assay for phosphatase activity.

In some examples, the membrane-associated protein is endogenous to a parent cell from which the BV is derived.

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

In another example, 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.

In some examples, the sample from the subject is a urine sample, a blood sample, or a digested tissue sample

In some examples, 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.

In some examples, the method further comprises flowing the culture medium comprising the BV over the column.

In some examples, the method further comprises eluting the BV.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A 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.