

making extracellular vesicles (EVs). The present disclosure also relates to novel EV-based ELISA assays and kits for performing such assays, as well as methods of producing antibodies to particular antigens using EVs comprising a membrane-bound antigen of interest.

In one aspectexample, the present disclosure provides methods for producing an antibody that specifically bind to a protein. In certain embodimentexamples, the method includes (a) producing a plurality of EVs comprising a heterologous protein by (i) expressing the heterologous protein in a cell exposed to a vesicle factor, (ii) culturing the cell in a medium and (iii) isolating the plurality of EVs comprising the heterologous protein from the medium, wherein the vesicle factor is selected from the group consisting of Acyl.Hrs, ARRDC1, ARF6 and a combination thereof; (b) immunizing an animal by administering the plurality of EVs to the animal; and (c) isolating an antibody that binds to the heterologous protein from the animal. In one aspect, the present disclosure provides a method for producing an antibody that specifically binds to a protein comprising: (a) producing a plurality of extracellular vesicles (EVs) comprising a heterologous protein by (i) expressing the heterologous protein in a cell exposed to a vesicle factor, (ii) culturing the cell in a medium and (iii) isolating the plurality of EVs comprising the heterologous protein from the medium, wherein the vesicle factor is selected from the group consisting of Acyl.Hrs, ARRDC1, ARF6 and a combination thereof; (b) immunizing an animal by administering the plurality of EVs to the animal; and (c) isolating an antibody that binds to the heterologous protein from the animal.

Alternatively and/or additionally, a method for producing an antibody that specifically binds to a protein can include (a) producing a plurality of EVs comprising a heterologous protein by (i) expressing the heterologous protein in a cell, (ii) culturing the cell in a medium and (iii) isolating the plurality of EVs comprising the protein from the medium, wherein the cell is a non-adherent cell; (b) immunizing an animal by administering the plurality of EVs to the animal; and (c) isolating an antibody that binds to the heterologous protein from the animal. In certain embodimentexamples, the method can further include expressing a vesicle factor, *e.g.*, a heterologous vesicle factor, in the cell, *e.g.*, MLGag, Acyl.Hrs, ARRDC1, ARF6 or a combination thereof.

In certain embodimentexamples, the plurality of EVs are isolated from the medium by ultracentrifugation. In certain embodimentexamples, the plurality of EVs is administered to the animal on week 0, week 2 and week 4. In certain embodimentexamples, the method for producing an antibody further comprises administering an adjuvant to the animal concurrently with the EVs, *e.g.*, a Ribi adjuvant. In certain embodimentexamples, the method for producing an antibody further comprises administering a boost to the animal to enhance an immune response in the animal to the protein. In certain embodimentexamples, the boost comprises the protein, a polynucleotide encoding the protein or a combination thereof.

The present disclosure further provides antibodies produced by the methods of the present disclosure. In one aspect, the present disclosure provides an isolated antibody or an antigen-binding portion thereof produced by the method as disclosed herein. In certain embodimentexamples, the antibody is a monoclonal antibody. In certain embodimentexamples, the antibody is a human, humanized or chimeric antibody. The

present disclosure further provides pharmaceutical compositions comprising the antibody or antigen-binding portion thereof and a pharmaceutically acceptable carrier. In one aspect, the present disclosure provides a pharmaceutical composition comprising the isolated antibody or antigen-binding portion thereof as disclosed herein and a pharmaceutically acceptable carrier. In certain ~~embodiment~~examples, the pharmaceutical composition further includes an additional therapeutic agent. In certain ~~embodiment~~examples, an antibody produced by the methods of the present disclosure, or pharmaceutical compositions thereof, can be used as a medicament, can be used in treating a disease and/or can be used in the manufacture of a medicament. In one aspect, the present disclosure provides the isolated antibody or antigen-binding portion thereof as disclosed herein for use as a medicament. In another aspect, the present disclosure provides the isolated antibody or antigen-binding portion thereof as disclosed herein for use in treating a disease. In another aspect, the present disclosure provides use of the isolated antibody or antigen-binding portion thereof as disclosed herein in the manufacture of a medicament. In yet another aspect, the present disclosure provides use of the isolated antibody or antigen-binding portion thereof as disclosed herein in the manufacture of a medicament for treating an individual having a disease. In one aspect, the present disclosure provides the pharmaceutical composition as disclosed herein for use in treating an individual having a disease. In certain ~~embodiment~~examples, the present disclosure provides methods of treating an individual having a disease, wherein the method includes administering to the individual an effective amount of an isolated antibody or antigen-binding portion thereof disclosed herein, or a pharmaceutical composition thereof. The present disclosure further provides isolated nucleic acids encoding an antibody or antigen-binding portion thereof disclosed herein, and host cells comprising the nucleic acid. In one aspect, the present disclosure provides an isolated nucleic acid encoding the antibody or antigen-binding portion as disclosed herein. In another aspect, the present disclosure provides a host cell comprising the nucleic acid as disclosed herein. The present disclosure also provides methods of producing an antibody by culturing the host cell under conditions suitable for expression of the antibody and, optionally, isolating the antibody from the host cell. In one aspect, the disclosure provides a method of producing an antibody or antigen-binding portion thereof comprising culturing the host cell as disclosed herein under conditions suitable for expression of the antibody.

In a further ~~aspect~~example, the present disclosure provides a method for producing a plurality of EVs. In certain ~~embodiment~~examples, the method includes (a) expressing a heterologous protein in a cell; (b) culturing the cell in a medium; and (c) isolating the plurality of EVs comprising the heterologous protein from the medium, wherein the cell is exposed to a vesicle factor selected from the group consisting of Acyl.Hrs, ARRDC1, ARF6 and a combination thereof, and/or wherein the cell is a non-adherent cell. In one aspect, the present disclosure provides a method for producing a plurality of extracellular vesicles (EVs) displaying a protein, comprising: (a) expressing a heterologous protein in a cell; (b) culturing the cell in a medium; and (c) isolating the plurality of EVs comprising the heterologous protein from the medium, wherein the cell is exposed to a vesicle factor selected from the group consisting of Acyl.Hrs, ARRDC1, ARF6 and a combination thereof.

In certain ~~embodiment~~examples, the heterologous protein is a membrane protein, e.g., a single-pass membrane protein or a multi-pass membrane protein. In certain ~~embodiment~~examples, the membrane protein is a member of a protein complex. In certain ~~embodiment~~examples, the membrane protein is not a transmembrane protein but is a member of a complex with a transmembrane protein. In certain ~~embodiment~~examples, the non-adherent cell is a 293S cell or an Expi293F™ cell.

In another ~~aspect~~example, the present disclosure provides methods for detecting an antibody. For example, but not by way of limitation, the method can include (a) incubating a sample with a capture reagent, wherein the capture reagent comprises a plurality of EVs comprising a membrane-bound antigen, and the antibody binds specifically to the membrane-bound antigen; and (b) contacting the antibody bound to the capture reagent with a detectable antibody to detect the bound antibody, wherein the detectable antibody binds specifically to the antibody. In certain ~~embodiment~~examples, the plurality of EVs are

generated by (i) expression of the membrane-bound antigen in a cell, (ii) culturing the cell *in vitro* in a medium to produce the plurality of EVs displaying the membrane-bound antigen and (iii) isolating the plurality of EVs displaying the membrane-bound antigen from the medium. In certain embodimentexamples, the cell is exposed to a vesicle factor selected from the group consisting of Acyl.Hrs, ARRDC1, ARF6 and a combination thereof and/or the cell is a non-adherent cell. In certain embodimentexamples, the method can further include (c) measuring the amount of the antibody detected in (b), wherein the amount is quantitated using a standard curve. In certain embodimentexamples, the sample is a plasma, serum or urine sample. In certain embodimentexamples, the capture antibody can be immobilized on a solid support, *e.g.*, a microtiter plate. In certain embodimentexamples, the detectable antibody is fluorescently labeled. In certain embodimentexamples, the membrane-bound antigen is a membrane protein or a fragment thereof.

The present disclosure provides kits for detecting an antibody in a sample.

In certain embodimentexamples, a kit of the present disclosure includes (a) a capture reagent that comprises a plurality of EVs comprising membrane-bound antigen, wherein the antibody to be detected binds specifically to the membrane-bound antigen; and (b) a detectable antibody binds specifically to the antibody to be detected. In certain embodimentexamples, the plurality of EVs are immobilized on a solid support. In certain embodimentexamples, the solid support is a microtiter plate. In certain embodimentexamples, the detectable antibody is fluorescently labeled. In certain embodimentexamples, the antigen is a membrane protein or a fragment thereof.

In certain embodimentexamples, the present disclosure further provides a method for sorting antibody-producing cells. In certain embodimentexamples, the method includes incubating the antibody-producing cells with a plurality of EVs wherein the plurality of EVs comprise: (i) a first population of EVs comprising a membrane-bound antigen and a first detectable marker, wherein a subset of the antibody-producing cells bind specifically to the membrane-bound antigen; and (b) a second population of EVs lacking the membrane-bound antigen but comprising a second detectable marker distinguishable from the first marker. In certain embodimentexamples, the method further includes sorting the antibody-producing cells based on their binding to either the first population of EVs or to a combination of the first population of EVs and the second population of EVs. In certain embodimentexamples, the first population of EVs is generated by (i) expression of the membrane-bound antigen and the first detectable marker in a first cell, (ii) culturing the first cell *in*

vitro in a medium to produce the plurality of EVs displaying the membrane-bound antigen and (iii) isolating the plurality of EVs displaying the membrane-bound antigen from the medium. In certain [embodimentexamples](#), the second population of EVs is generated by (i) expression of the second detectable marker in a second cell, (ii) culturing the second cell *in vitro* in a medium to produce the plurality of EVs comprising the second detectable marker and (iii) isolating the plurality of EVs from the medium. In certain [embodimentexamples](#), the cell is exposed to a vesicle factor selected from the group consisting of Acyl.Hrs, ARRDC1, ARF6 and a combination thereof. In certain [embodimentexamples](#), the first cell and/or second cell is a non-adherent cell.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. A schematic drawing showing the formation of extracellular vesicles (EVs).

Figs. 2A-2B. Design principles for EV formation. (2A) General EV former and Acyl.Hrs possessing EV designs. (2B) MLGag and ARRDC1 possessing EV designs.

Fig. 3. A schematic drawing showing the workflow of producing EVs.

Fig. 4. Identification of vesicle factors that can produce EVs expressing MP-X using Western Blot.

Fig. 5. Dynamic Light Scattering (DLS) showing uniform vesicle sizes.

Fig. 6. Western Blot showed vesicle factors MLGag, Acyl.Hrs and murine ARRDC1 (mARRDC1) induced MP-X-expressing EV formation in murine cells.

Figs. 7A-7B. EV production challenges: (7A) challenges in obtaining efficient EV purification; and (7B) challenges in obtaining sufficient yield.

Figs. 8A-8C. Average yields of EVs (8A, 8B) and cell viability (8C) were measured on harvest day in Expi293FTM cells and 293S cells, which were used for producing EVs expressing target proteins, having MLGag as the vesicle factor.

Figs. 9A-9C. ELISA was performed to compare the expression levels of MP-7 (9A), MP-8 (9B), or MP-4 (9C) between the Expi293FTM cell produced EVs and 293S cell produced EVs.

Figs. 10A-10C. (10A) Well-defined EV particles were generated. (10B, 10C) EV-based ELISA can detect FACS+ antibodies against single pass (10B) and multi-pass (10C) membrane proteins.