

intra-membrane region is situated at the 5' or 3' end of the first nucleic acid fragment; and
(b) a second nucleic acid fragment that encodes a fowlpox virus (FPV) FPV108 protein or functional fragment thereof, wherein the second nucleic acid fragment is fused in frame to a portion of the first nucleic acid fragment that encodes an intra-membrane region of the IMP; wherein a poxvirus infected cell comprising the polynucleotide can express an IMP-FPV108 fusion protein as part of the outer envelope membrane of an extracellular enveloped virion (EEV).

[0004b] In one aspect, the disclosure provides an FPX108 fusion protein encoded by the polynucleotide as disclosed herein. In another aspect, the disclosure provides a poxvirus genome comprising the polynucleotide as disclosed herein. In another aspect, the disclosure provides a recombinant poxvirus EEV comprising the poxvirus genome as disclosed herein.

[0004c] In one aspect, the disclosure provides an *in vitro* method of producing the recombinant poxvirus EEV as disclosed herein, comprising: (a) infecting a host cell permissive for vaccinia virus, fowlpox virus, or rabbit pox virus infectivity with a vaccinia virus, fowlpox virus or rabbit pox virus, respectively, wherein the vaccinia virus comprises the poxvirus genome as disclosed herein, the fowlpox virus comprises the poxvirus genome as disclosed herein, and the rabbit pox virus comprises the poxvirus genome as disclosed herein; and (b) recovering EEV released from the host cell.

[0004d] In one aspect, the disclosure provides an *in vitro* method to display an integral membrane protein (IMP) or fragment thereof in a native conformation comprising: (a) infecting host cells permissive for poxvirus infectivity with a recombinant poxvirus that expresses the IMP or fragment thereof as a fusion protein with the EEV-specific protein FPV108 or RBX041 or a membrane-associated fragment thereof, wherein EEV produced by the infected host cell comprise the IMP fusion protein as part of the EEV outer envelope membrane; (b) recovering EEV released from the host cell wherein the IMP or fragment thereof displays on the surface of the EEV in a native conformation.

[0004e] In one aspect, the disclosure provides a fusion protein comprising: (a) the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 11, wherein the fusion protein, when expressed by a recombinant poxvirus, appears on the surface of a poxvirus extracellular enveloped virion (EEV) in a native conformation.

[0004f] In one aspect, the disclosure provides a recombinant poxvirus EEV comprising the fusion protein as disclosed herein. In other aspect, the disclosure provides a recombinant poxvirus EEV comprising a heterologous IMP or fragment thereof fused to a fowlpox virus FPV108 protein or membrane-associated fragment thereof, wherein the fusion protein is situated in the EEV outer envelope membrane, wherein the IMP or fragment thereof displays on the surface of the EEV in its native conformation.

[0004g] In another aspect, the disclosure provides a method to select antibodies that bind to a multi-pass membrane protein (IMP) comprising: (a) attaching the recombinant EEV as described herein to a solid support; (b) providing an antibody display library, wherein the library comprises display packages displaying a plurality of antigen binding domains; (c) contacting the display library with the EEV such that display packages displaying antigen binding domains that specifically binds to the IMP expressed on the EEV can bind thereto; (d) removing unbound display packages; and (e) recovering display packages that display an antigen binding domain specific for the IMP expressed on the EEV.

[0004h] In another aspect, the disclosure provides a method to select antibodies that bind to a multi-pass membrane protein (IMP) comprising: (a) providing a first and second recombinant poxvirus EEV of claim 23, wherein the first and second recombinant poxvirus EEV are each generated in a different poxvirus, wherein an animal is to be immunized with the first recombinant poxvirus; (b) contacting a display library that comprises display packages displaying a plurality of antigen binding domains with the second recombinant poxvirus such that the display packages displaying antigen binding domains that specifically bind to the IMP expressed on the EEV can bind thereto, wherein said display library is generated from B cells which are to be isolated from the immunized animal; (c) removing

What is claimed is:

1. An isolated polynucleotide comprising: (a) a first nucleic acid fragment that encodes an integral membrane protein (IMP) or fragment thereof, wherein the IMP or fragment thereof comprises at least one extra-membrane region, at least one transmembrane domain and at least one intra-membrane region, and wherein a portion of the first nucleic acid fragment encoding at least one intra-membrane region is situated at the 5' or 3' end of the first nucleic acid fragment; and (b) a second nucleic acid fragment that encodes a fowlpox virus (FPV) FPV108 protein or functional fragment thereof, wherein the second nucleic acid fragment is fused in frame to a portion of the first nucleic acid fragment that encodes an intra-membrane region of the IMP; wherein a poxvirus infected cell comprising the polynucleotide can express an IMP-FPV108 fusion protein as part of the outer envelope membrane of an extracellular enveloped virion (EEV).
2. The polynucleotide of claim 1, wherein the second nucleic acid encodes FPV108 protein comprising the amino acid sequence SEQ ID NO: 2 or a functional fragment thereof.
3. The polynucleotide of claim 1, wherein the IMP is a multi-pass membrane protein comprising at least two transmembrane domains.
4. The polynucleotide of claim 3, wherein the IMP has an odd number of transmembrane domains, wherein the 5' end of the first nucleic acid fragment encodes an extra-membrane region, wherein the 3' end of the first nucleic acid fragment encodes an intra-membrane region, and wherein the 5' end of the second polynucleotide is fused to the 3' end of the first nucleic acid fragment.
5. The polynucleotide of claim 4, wherein the IMP comprises a G-protein coupled receptor (GPCR).
6. The polynucleotide of claim 4, wherein the IMP is the human frizzled-4 protein (FZD4) or a

CXC chemokine receptor (CXCR), or a fragment thereof.

7. The polynucleotide of claim 4, wherein the IMP is the CXC chemokine receptor CXCR4, or a fragment thereof.

8. The polynucleotide of claim 1, wherein the IMP has an even number of transmembrane domains, and wherein both the 5' and 3' ends of the first nucleic acid fragment encode intra-membrane regions, and wherein the second nucleic acid fragment is fused to 3' end of the first nucleic acid fragment.

9. The polynucleotide of claim 8, wherein the IMP is human CD20 or CD39 protein, or a fragment thereof.

10. The polynucleotide of any one of claims 1 to 9, which is operably associated with a poxvirus promoter.

11. The polynucleotide of any one of claims 1 to 10, wherein the first and second nucleic acid fragments are directly fused.

12. The polynucleotide of any one of claims 1 to 11, further comprising a third nucleic acid fragment encoding a heterologous peptide.

13. The polynucleotide of claim 12, wherein the heterologous peptide comprises a linker sequence, an amino acid tag or label, or a peptide or polypeptide sequence that facilitates purification.

14. The polynucleotide of any one of claims 1 to 13, which is operably associated with a poxvirus promoter.

15. The polynucleotide of claim 14, wherein the poxvirus promoter is p7.5 or H5 or T7.

16. An FPX108 fusion protein encoded by the polynucleotide of any one of claims 1 to 15.
17. A poxvirus genome comprising the polynucleotide of any one of claims 1 to 15.
18. The poxvirus genome of claim 17, which is a vaccinia virus genome.
19. The poxvirus genome of claim 17, which is a fowlpox virus genome.
20. The poxvirus genome of claim 17, which is a rabbit pox virus genome.
21. A recombinant poxvirus EEV comprising the poxvirus genome of any one of claims 17 to 20.
22. An *in vitro* method of producing the recombinant poxvirus EEV of claim 21, comprising: (a) infecting a host cell permissive for vaccinia virus, fowlpox virus, or rabbit pox virus infectivity with a vaccinia virus, fowlpox virus or rabbit pox virus, respectively, wherein the vaccinia virus comprises the poxvirus genome of claim 18, the fowlpox virus comprises the poxvirus genome of claim 19, and the rabbit pox virus comprises the poxvirus genome of claim 20; and (b) recovering EEV released from the host cell.
23. An *in vitro* method to display an integral membrane protein (IMP) or fragment thereof in a native conformation comprising: (a) infecting host cells permissive for poxvirus infectivity with a recombinant poxvirus that expresses the IMP or fragment thereof as a fusion protein with the EEV-specific protein FPV108 or a membrane-associated fragment thereof, wherein EEV produced by the infected host cell comprise the IMP fusion protein as part of the EEV outer envelope membrane; (b) recovering EEV released from the host cell wherein the IMP or fragment thereof displays on the surface of the EEV in a native conformation.
24. The *in vitro* method of claim 23, wherein the IMP is a multi-pass membrane protein comprising at least two transmembrane domains.
25. The *in vitro* method of claim 24, wherein the IMP comprises a G-protein coupled receptor

(GPCR) comprising seven transmembrane domains, and wherein FPV108 is fused to the C-terminus of the IMP.

26. The *in vitro* method of claim 25, wherein the IMP is the human frizzled-4 protein (FZD4), a CXC chemokine receptor or a fragment thereof.

27. The *in vitro* method of claim 26, wherein the IMP is the CXC chemokine receptor CXCR4, or a fragment thereof.

28. The *in vitro* method of any one of claims 23 or 24, wherein the IMP or fragment thereof has an even number of transmembrane domains, and wherein both the N-terminus and the C-terminus of the IMP or fragment thereof are intra-membrane.

29. The *in vitro* method of claim 28, wherein FPV108 is fused to the C-terminus of the IMP.

30. The *in vitro* method of claim 29, wherein the IMP is human CD20, or a fragment thereof.

31. A fusion protein comprising: (a) the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 11, wherein the fusion protein, when expressed by a recombinant poxvirus, appears on the surface of a poxvirus extracellular enveloped virion (EEV) in a native conformation.

32. A recombinant poxvirus EEV comprising the fusion protein of claim 31.

33. A recombinant poxvirus EEV comprising a heterologous IMP or fragment thereof fused to a fowlpox virus FPV108 protein or membrane-associated fragment thereof, wherein the fusion protein is situated in the EEV outer envelope membrane, wherein the IMP or fragment thereof displays on the surface of the EEV in its native conformation.

34. The recombinant poxvirus EEV of claim 33, wherein the poxvirus EEV is a fowlpox virus EEV.

35. A method to select antibodies that bind to a multi-pass membrane protein (IMP) comprising: (a) attaching the recombinant EEV of any one of claims 21, 32, 33, or 34 to a solid support; (b) providing an antibody display library, wherein the library comprises display packages displaying a plurality of antigen binding domains; (c) contacting the display library with the EEV such that display packages displaying antigen binding domains that specifically binds to the IMP expressed on the EEV can bind thereto; (d) removing unbound display packages; and (e) recovering display packages that display an antigen binding domain specific for the IMP expressed on the EEV.

36. A method to select antibodies that bind to a multi-pass membrane protein (IMP) comprising: (a) providing a first and second recombinant poxvirus EEV of claim 21, wherein the first and second recombinant poxvirus EEV are each generated in a different type of poxvirus, wherein an animal is to be immunized with the first recombinant poxvirus; (b) contacting a display library that comprises display packages displaying a plurality of antigen binding domains with the second recombinant poxvirus such that the display packages displaying antigen binding domains that specifically bind to the IMP expressed on the EEV can bind thereto, wherein said display library is generated from B cells which are to be isolated from the immunized animal; (c) removing unbound display packages; and (d) recovering display packages that display an antigen binding domain specific for the IMP expressed on the second recombinant EEV.

37. The method of claim 36, wherein the first recombinant poxvirus EEV is a vaccinia virus EEV or a fowlpox virus EEV.

38. A method to select antibodies or antigen-binding fragments thereof that bind to a multi-pass membrane protein (IMP), which comprises: (a) providing a recombinant poxvirus EEV of claim 21, wherein an animal is to be immunized with the recombinant poxvirus EEV; wherein optionally the animal is to be immunized with a second dose of the recombinant poxvirus EEV; (b) isolating serum from the immunized animal; and (c) isolating antibodies or antigen-binding fragments thereof that comprise an antigen binding domain specific for the IMP expressed on the recombinant poxvirus EEV.