WHAT IS CLAIMED IS:

1. An isolated extracellular vesicle (EV) comprising a biologically active molecule linked to a scaffold protein,

wherein the scaffold protein comprises an N-terminus domain (ND) and an effector domain (ED),

wherein the ND is associated with the luminal surface of the EV and the ED is associated with the luminal surface of the EV,

wherein the ND comprises the amino acid sequence GGKLSK (SEQ ID NO: 203), but does not comprise a methionine (Met) at the N-terminus,

wherein the ED comprises a lysine (Lys) at its N-terminus that is linked directly to the lysine at the C-terminus of SEQ ID NO: 203 in the ND; and

wherein the biologically active molecule comprises one or more heterologous proteins fused to the C-terminus of the scaffold protein.

2. The EV of claim 1, wherein the ED comprises KK, KKK, KKKK (SEQ ID NO: 151), KKKKK (SEQ ID NO: 152), or any combination thereof.

3. The EV of claim 1 or 2, wherein the scaffold protein is linked to the biologically active molecule by a linker.

4. The EV of claim 3, wherein the linker comprises a cleavable linker.

5. The EV of any one of claims 1 to 4, wherein the scaffold protein comprises GGKLSKKK (SEQ ID NO: 161) or GGKLSKKS (SEQ ID NO: 162).

6. The EV of any one of claims 1 to 5, wherein the scaffold protein is at least about 8 amino acids in length.

7. The EV of any one of claims 1 to 6, wherein the biologically active molecule is on the luminal surface or in the lumen of the EV.

8. The EV of any one of claims 1 to 7, wherein the scaffold protein further comprises:

(i) a transmembrane domain,

(ii) an extravesicular domain, or

(iii) both (i) and (ii).

9. The EV of any one of claims 1 to 6, wherein the scaffold protein further comprises a transmembrane domain and an extravesicular domain and:

(i) the transmembrane domain is between the ED domain of the scaffold protein and the biologically active molecule, and

(ii) the biologically active molecule is linked to the extravesicular domain.

10. The EV of any one of claims 1 to 9, wherein the biologically active molecule comprises:
(a) a protein, wherein the protein optionally comprises

(i) a recombinant peptide, a natural peptide, a synthetic peptide, an antibody, a fusion protein, or any combination thereof;

(ii) an enzyme, a cytokine, a ligand, a receptor, a transcription factor, or a combination thereof;

(iii) a T-cell receptor (TCR), a T-cell co-receptor, a major histocompatibility complex (MHC), a human leukocyte antigen (HLA), or a derivative thereof;

(iv) a tumor antigen optionally selected from the group consisting of alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), epithelial tumor antigen (ETA), mucin 1 (MUC1), Tn-MUC1, mucin 16 (MUC16), tyrosinase, melanoma-associated antigen (MAGE), tumor protein p53 (p53), CD4, CD8, CD45, CD80, CD86, programmed death ligand 1 (PD-L1), programmed death ligand 2 (PD-L2), NY-ESO-1, PSMA, TAG72, HER2, GD2, cMET, EGFR, Mesothelin, VEGFR, alpha-folate receptor, CE7R, IL-3, Cancer-testis antigen, MART-1 gp100, and TNF-related apoptosis-inducing ligand;

(b) a polypeptide;
(c) a peptide;
(d) a polynucleotide (DNA and/or RNA);
(e) a chemical compound;
(f) a virus, wherein the virus optionally comprises an adeno-associated virus, a parvovirus, a retrovirus, an adenovirus, or any combination thereof;
(g) an ionophore;
(h) a carrier for an ionophore;
(i) a moiety that forms a channel or a pore; or
(j) any combination thereof.

11. The EV of any one of claims 1 to 10, wherein the EV further comprises a second scaffold protein, wherein the second scaffold protein optionally comprises a PTGFRN polypeptide, a BSG polypeptide, an IGSF2 polypeptide, an IGSF3 polypeptide, an IGSF8 polypeptide, an ITGB1 polypeptide, an ITGA4 polypeptide, a SLC3A2 polypeptide, an ATP transporter polypeptide, an aminopeptidase N (ANPEP) polypeptide, an ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (ENPP1) polypeptide, a neprilysin (MME) polypeptide, a neuropilin-1 (NRP1) polypeptide, or a fragment thereof.

12. The EV of any one of claims 1 to 11, wherein the biologically active molecule is:
(a) an inhibitor for a negative checkpoint regulator or an inhibitor for a binding partner of a negative checkpoint regulator, wherein the negative checkpoint regulator is optionally selected from the group consisting of:

(i) cytotoxic T-lymphocyte-associated protein 4 (CTLA-4),
(ii) programmed cell death protein 1 (PD-1),
(iii) lymphocyte-activated gene 3 (LAG-3),
(iv) T-cell immunoglobulin mucin-containing protein 3 (TIM-3),
(v) B and T lymphocyte attenuator (BTLA),
(vi) T cell immunoreceptor with Ig and ITIM domains (TIGIT),
(vii) V-domain Ig suppressor of T cell activation (VISTA),
(viii) adenosine A2a receptor (A2aR),
(ix) killer cell immunoglobulin like receptor (KIR),
(x) indoleamine 2,3-dioxygenase (IDO),
(xi) CD20,
(xii) CD39, and
(xiii) CD73;

(b) an immunogenic protein;

(c) a toxin, toxoid, or a non-toxic mutant of a toxin, wherein

(i) the toxin is optionally a diphtheria toxin, or
(ii) the toxoid is optionally a tetanus toxoid;

(d) an activator for a positive co-stimulatory molecule or an activator for a binding partner of a positive costimulatory molecule, wherein the positive co-stimulatory molecule is optionally selected from the group consisting of:

(i) a TNF receptor superfamily member optionally selected from the group consisting of: CD120a, CD120b, CD18, OX40, CD40, Fas receptor, M68, CD27, CD30, 4-1BB, TRAILR1, TRAILR2, TRAILR3, TRAILR4, RANK, OCIF, TWEAK receptor, TACI, BAFF receptor, ATAR, CD271, CD269, AITR, TROY, CD358, TRAMP, and XEDAR, optionally wherein the activator for the positive co-stimulatory molecule is a TNF superfamily member optionally selected from the group consisting of: TNFα, TNF-C, OX40L, CD40L, FasL, LIGHT, TL1A, CD27L, Siva, CD153, 4-1BB ligand, TRAIL, RANKL, TWEAK, APRIL, BAFF, CAMLG, NGF, BDNF, NT-3, NT-4, GITR ligand, and EDA-2; and

(ii) a CD28-superfamily co-stimulatory molecule optionally selected from ICOS and CD28, optionally wherein the activator for the positive co-stimulatory molecule is ICOSL, CD80 or CD86.

13. The EV of any one of claims 1 to 12, wherein the EV is an exosome.

14. A pharmaceutical composition comprising the EV of any one of claims 1 to 13 and a pharmaceutically acceptable carrier.

15. Use of the EV of any one of claims 1 to 13 in the manufacture of medicament for the prevention or treatment of a disease in a subject in need thereof.