Claims

What is claimed is:

1. A minimal arrestin domain-containing protein 1 (ARRDC1) comprising:
an arrestin domain,
at least one PSAP (SEQ ID NO: 122) or PTAP (SEQ ID NO: 123) motif, and
at least one PPXY motif, wherein the minimal ARRDC1 is shorter than full-length ARRDC1 protein.

2. The minimal ARRDC1 of claim 1, wherein the ARRDC1 comprises at least two PPXY motifs.

3. The minimal ARRDC1 of claims 1 or 2, wherein the minimal ARRDC1 is less than 400 amino acids in length.

4. The minimal ARRDC1 of any of claims 1-3, wherein the minimal ARRDC1 is less than 350 amino acids in length.

5. The minimal ARRDC1 of any of claims 1-3, wherein the at least one PPXY motif is PPEY (SEQ ID NO: 124).

6. The minimal ARRDC1 of any of claims 1-3, wherein the at least one PPXY motif is PPSY(SEQ ID NO: 125).

7. The minimal ARRDC1 of claim 2, wherein the at least two PPXY motifs are PPEY(SEQ ID NO: 124). and PPSY(SEQ ID NO: 125).

8. The minimal ARRDC1 of any of claims 1-7, wherein the minimal ARRDC1 comprises an amino acid sequence that is at least 85% identical, or optionally 90% identical, or optionally 95% identical to the amino acid sequence set forth in SEQ ID NO: 1.

9. The minimal ARRDC1 of any of claims 1-8, wherein the minimal ARRDC1 comprises the amino acid sequence set forth in SEQ ID NO: 1.

10. An arrestin domain-containing protein 1 (ARRDC1)-mediated microvesicle (ARMM), comprising:
a lipid bilayer and a minimal ARRDC1 protein or variant thereof, wherein the ARRDC1 protein comprises at least one PSAP (SEQ ID NO: 122) or PTAP (SEQ ID NO: 123) motif and at least one PPXY motif, and wherein the ARRDC1 protein is shorter than full-length ARRDC1 protein.

11. The microvesicle of claim 10, wherein the minimal ARRDC1 comprises at least two PPXY motifs.

12. The microvesicle of claims 10 or 11, wherein the minimal ARRDC1 protein comprises the amino acid sequence set forth in SEQ ID NO: 1.

13. The microvesicle of any of claims 10-12, further comprising an agent.

14. The microvesicle of claim 13, wherein the agent is selected from the group consisting of a nucleic acid, a protein, and a small molecule.

15. The microvesicle of any one of claims 10-14, wherein the microvesicle further comprises a TSG101 protein or fragment thereof.

16. The microvesicle of claim 15, wherein the TSG101 protein fragment comprises a TSG101 UEV domain.

17. The microvesicle of any of claims 10-16, wherein the agent is conjugated to, or expressed as a fusion protein with, the minimal ARRDC1 protein, the minimal ARRDC1 fragment, the TSG101 protein, or the TSG101 fragment.

18. The microvesicle of any one of claims 10-17, wherein the microvesicle further comprises an integrin, a receptor tyrosine kinase, a G-protein coupled receptor, or a membrane-bound immunoglobulin.

19. The microvesicle of any one of claims 10-18, wherein the microvesicle comprises an agent selected from the group consisting of Cas9 protein or Cas9 protein variant, Oct4, Sox2,

c-Myc, and KLF4 reprogramming factor, p53, Rb (retinoblastoma protein), BRCA1, BRCA2, PTEN, APC, CD95, ST7, ST14, a BCL-2 family protein, a caspase; BRMS1, CRSP3, DRG1, KAI1, KISS1, NM23, a TIMP-family protein, a BMP-family growth factor, EGF, EPO, FGF, G-CSF, GM-CSF, a GDF-family growth factor, HGF, HDGF, IGF, PDGF, TPO, TGF-α, TGF-β, VEGF; a zinc finger nuclease, Cre, Dre, FLP recombinase, , Hin, Gin, Tn3, β-six, CinH, ParA, γδ, Bxb1, ϕC31, TP901, TG1, φBT1, R4, φRV1, φFC1, MR11, A118, U153, gp29, Cre, FLP, R, Lambda, HK101, HK022, pSAM2, CAS9 nuclease, Sp1, NF1, CCAAT, GATA, HNF, PIT-1, MyoD, Myf5, Hox, Winged Helix, SREBP, p53, CREB, AP-1, Mef2, STAT, R-SMAD, NF-κB, Notch, TUBBY, NFAT, α1β1 integrin, α2β1 integrin, α4β1 integrin, α5β1 integrin, α6β1 integrin, αLβ2 integrin, αMβ2 integrin, αIIbβ3 integrin, αVβ3 integrin, αVβ5 integrin, αVβ6 integrin, α6β4 integrin, EGF receptor (ErbB family), insulin receptor, PDGF receptor, FGF receptor, VEGF receptor, HGF receptor, Trk receptor, Eph receptor, AXL receptor, LTK receptor, TIE receptor, ROR receptor, DDR receptor, RET receptor, KLG receptor, RYK receptor, MuSK receptor, rhodopsin-like receptor, the secretin receptor, metabotropic glutamate/pheromone receptor, cyclic AMP receptor, frizzled/smoothened receptor, CXCR4 receptor, CCR5 receptor, beta-adrenergic receptor, CA19-9, c-met, PD-1, CTLA-4, ALK, AFP, EGFR, Estrogen receptor (ER), Progesterone receptor (PR), HER2/neu, KIT, B-RAF, S100, MAGE, Thyroglobulin, MUC-1, and PSMA.

20. The microvesicle of any of claims 14-19, wherein the nucleic acid comprises an RNA.

21. The microvesicle of any of claims 14-20, wherein the nucleic acid comprises an RNAi agent.

22. The microvesicle of any of claims 14-20, wherein the nucleic acid comprises a coding RNA, a non-coding RNA, an antisense RNA, an mRNA, a small RNA, an siRNA, an shRNA, a microRNA, an snRNA, a snoRNA, a lincRNA, a structural RNA, a ribozyme, or a precursor thereof.

23. The microvesicle of any of claims 14-19, wherein the nucleic acid comprises a DNA.

24. The microvesicle of claim 23, wherein the DNA comprises a restrotransposon sequence, a LINE sequence, a SINE sequence, a composite SINE sequence, or an LTR-retrotransposon sequence.

25. The microvesicle of any of claims 14-24, wherein the nucleic acid encodes a protein.

26. The microvesicle of any of claims 13-25, wherein the agent comprises a detectable label.

27. The microvesicle of any of claims 13-26, wherein the agent comprises a therapeutic agent.

28. The microvesicle of claim 27, wherein the agent is selected from the group consisting of an enzyme, an antibody, a Fab, a Fab', a F(ab')2, a Fd, a scFv, a Fv, a dsFv, a diabody, and an affibody.

29. The microvesicle of any of claims 13-28, wherein the agent comprises a cytotoxic agent.

30. The microvesicle of any of claims 13-29, wherein the agent comprises a protein.

31. The microvesicle of claim 30, wherein the agent comprises a transcription factor, a transcriptional repressor, a fluorescent protein, a kinase, a phosphatase, a protease, a ligase, or a recombinase.

32. The microvesicle of any of claims 13 -31, wherein the agent is covalently bound to the minimal ARRDC1 protein or fragment thereof, or the TSG101 protein or fragment thereof.

33. The microvesicle of any of claims 13-32, wherein the agent is conjugated to the minimal ARRDC1 protein or fragment thereof or the TSG101 protein or fragment thereof via a linker.

34. The microvesicle of claim 33 wherein the linker is a cleavable linker.

35. The microvesicle of claim 34 wherein the linker comprises a protease recognition site or a UV-cleavable moiety.

36. The microvesicle of claim 13-31, wherein the agent is fused to at least one WW domain or variant thereof.

37. The microvesicle of claim 36, wherein the agent comprises two, three, four, or five WW domains or variants thereof.

38. The microvesicle of claims 36 or 37, wherein the WW domain is derived from a WW domain of the ubiquitin ligase WWP1, WWP2, Nedd4-1, Nedd4-2, Smurf1, Smurf2, ITCH, NEDL1, or NEDL2.

39. The microvesicle of any of claims 36-38, wherein the WW domain comprises a sequence selected from the group consisting of SEQ ID NO: 6-14.

40. The microvesicle of any of claims 36-39, wherein the agent is a protein, optionally wherein the agent is a fusion protein.

41. The microvesicle of any of claims 36-40, wherein the agent is Cas9 protein.

42. The microvesicle of claim 41, wherein the Cas9 protein or variant thereof comprises at least one nuclear localization sequence (NLS).

43. The microvesicle of claims 41 or 42 further comprising a guide RNA (gRNA).

44. The microvesicle of any of claims 36-43, wherein the WW domain is fused to the N-terminus of the protein.

45. The microvesicle of any of claims 36-44, wherein the WW domain is fused to the C-terminus of the protein.

46. The microvesicle of any of claims 10-45, wherein the microvesicle does not include an exosomal biomarker.

47. The microvesicle of any of claims 10-46, wherein the microvesicle is negative for an exosomal biomarker.

48. The microvesicle of claim 46 or 47, wherein the exosomal biomarker is chosen from the group consisting of CD63, Lamp-1, Lamp-2, CD9, HSPA8, GAPDH, CD81, SDCBP, PDCD6IP, ENO1, ANXA2, ACTB, YWHAZ, HSP90AA129, ANXA5, EEF1A1, YWHAE, PPIA, MSN, CFL1, ALDOA, PGK1, EEF2, ANXA1, PKM2, HLA-DRA, and YWHAB.

49. The microvesicle of any of claims 46-48, wherein the microvesicle does not include, or is negative for CD63 and Lamp-1.

50. The microvesicle of any of claims 10- 49, wherein the microvesicle diameter is from about 30 nm to about 500 nm.

51. An arrestin domain-containing protein 1 (ARRDC1)-mediated microvesicle (ARMM), comprising:
a lipid bilayer;
a minimal ARRDC1 protein or variant thereof, wherein the ARRDC1 protein comprises an arrestin domain, at least one PSAP (SEQ ID NO: 122) or PTAP (SEQ ID NO: 123) motif, and at least two PPXY motifs, and wherein the ARRDC1 protein is shorter than full-length ARRDC1 protein; and
a Cas9 cargo protein, wherein the Cas9 cargo protein is linked to the minimal ARRDC1 protein.

52. The microvesicle of claim 51, wherein the minimal ARRDC1protein is covalently linked to the Cas9 cargo protein.

53. The microvesicle of claims 51 or 52, wherein the minimal ARRDC1protein is linked to the Cas9 protein via a cleavable linker.

54. The microvesicle of any of claims 51-53, wherein the linker comprises a protease recognition site.

55. The microvesicle of any of claims 51-54, wherein the linker comprises a UV-cleavable linker.

56. An arrestin domain-containing protein 1 (ARRDC1)-mediated microvesicle (ARMM), comprising:

a lipid bilayer;

a minimal ARRDC1 protein, wherein the minimal ARRDC1 protein comprises an arrestin domain, at least one PSAP (SEQ ID NO: 122) and/or PTAP (SEQ ID NO: 123) motif, and at least two PPXY motifs, and wherein the minimal ARRDC1 protein is shorter than full-length ARRDC1 protein;

a TSG101 protein or variant thereof; and

a Cas9 protein, wherein the Cas9 protein is linked to the TSG101 protein or variant thereof.

57. A minimal ARRDC1 fusion protein comprising:

a minimal ARRDC1 protein or a variant thereof, wherein the minimal ARRDC1 protein comprises an arrestin domain, at least one PSAP (SEQ ID NO: 122) motif, and at least two PPXY motifs, and wherein the minimal ARRDC1 protein is shorter than full-length ARRDC1 protein; and

a Cas9 protein or a variant thereof.

58. A microvesicle-producing cell comprising:

a recombinant expression construct encoding a minimal ARRDC1 protein of any of claims A1-A7 under the control of a heterologous promoter, and

a recombinant expression construct encoding a cargo protein under the control of a heterologous promoter.

59. The microvesicle-producing cell of claim 58, wherein the cargo protein is fused to at least one WW domain or variant thereof.

60. A microvesicle-producing cell comprising:

a recombinant expression construct encoding a minimal ARRDC1 protein under the control of a heterologous promoter, wherein the minimal ARRDC1 protein comprises an arrestin domain, at least one PSAP (SEQ ID NO: 122) and/or PTAP (SEQ ID NO: 123) motif, and at least two PPXY motifs, and wherein the minimal ARRDC1 protein is shorter than full-length ARRDC1 protein; and wherein the minimal ARRDC1 protein is linked to a Cas9 cargo protein or variant thereof.

61. A method of delivering a cargo to a target cell, the method comprising contacting the target cell with the microvesicle of any of claims 10-56.

62. A method of delivering a cargo to a target cell, the method comprising contacting the target cell with the microvesicle-producing cell of any of claims 58-60.

63. A method of gene editing comprising contacting the target cell with the microvesicle of any of claims 10-56.

64. A method of gene editing comprising contacting the target cell with the microvesicle-producing cell of any of claims 58-60.

65. A nucleic acid comprising a nucleotide sequence encoding the minimal arrestin domain-containing protein 1 (ARRDC1) of any of claims 1-8.