

CLAIMS

1. A fusion protein, comprising:
 - a protein of interest to be delivered to a cell;
 - a cell penetrating peptide (CPP); and
 - 5 a target enhancing sequence (TES).
2. The fusion protein of claim 1, wherein the CPP is located at the N-terminus of the fusion protein and wherein the TES is fused at the C-terminus of the CPP.
3. The fusion protein of claim 1 or 2, comprising or consisting of, starting at the N-terminus:
 - 10 CPP;
 - TES; and
 - a protein of interest.
4. The fusion protein of claim 1, wherein the CPP is located at the C-terminus of the fusion protein and wherein the TES is fused at the N-terminus of the CPP.
- 15 5. The fusion protein of claim 1 or 4, comprising or consisting of, starting at the C-terminus:
 - a protein of interest;
 - TES; and
 - CPP.
- 20 6. The fusion protein of any one of claims 1-5, wherein the protein of interest lacks an OTS.
7. The fusion protein of claim 6, wherein the fusion protein further comprises an organelle targeting sequence (OTS) exogenous to the protein of interest.

8. The fusion protein of any one of claims 1-5, wherein the protein of interest comprises an OTS.
9. The fusion protein of claim 8, wherein the OTS is heterologous to the protein of interest.
10. The fusion protein of claim 8, wherein the OTS is endogenous to the protein of interest.
- 5 11. The fusion protein of any one of claims 1-5, wherein the protein of interest is selected from the group consisting of Frataxin (FXN), Tafazin, Pyruvate Dehydrogenase (PDH), SLIRP, LRPPC, PARK2 protein, PARK6 protein, PARK7 protein, Phospholipase A2, Group VI and a variant or derivative thereof.
12. The fusion protein of claim 11, wherein the protein of interest comprises Frataxin (FXN)
10 or PARK2 protein, or a variant or derivative thereof.
13. The fusion protein of any one of claims 1-12, wherein the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0.
14. The fusion protein of any one of claims 1-13, wherein the CPP comprises a peptide selected from the group consisting of HIV-TAT, galanin, mastoparan, transportan, penetratin,
15 polyarginine, VP22, and a variant or derivative thereof.
15. The fusion protein of claim 14, wherein the CPP comprises HIV-TAT or a variant or derivative thereof.
16. The fusion protein of any one of claims 1-15, wherein the TES is a nuclear export signal peptide.
- 20 17. The fusion protein of claim 16, wherein the nuclear export signal peptide comprises a sequence having at least 85% sequence identity to any one of SEQ ID NOs. 36-43.
18. The fusion protein of claim 17, wherein the nuclear export signal peptide comprises a peptide selected from the group consisting of NES1, NES2, NES3, NES4, NES5, NES6, NES7, NES8, and a variant or derivative thereof.
- 25 19. The fusion protein of claim 18, wherein the nuclear export signal peptide comprises a peptide selected from the group consisting of NES1 and NES2, or a variant or derivative thereof.

20. The fusion protein of any one of claims 1-19, wherein the TES is a protease sensitive peptide.
21. The fusion protein of claim 20, wherein the protease sensitive peptide comprises a ubiquitin-like modifier.
- 5 22. The fusion protein of claim 20, wherein the protease sensitive peptide comprises a sequence having at least 85% sequence identity to any one of SEQ ID NOs. 18-31.
23. The fusion protein of claim 20, wherein the protease sensitive peptide comprises a peptide selected from the group consisting of ubiquitin, a caspase cleavage domain, a calpain cleavage domain, SUMO1, SUMO2, SUMO3, SUMO4, ISG15, Atg8, Atg12, NEDD8, and a
10 variant or derivative thereof.
24. The fusion protein of claim 1, wherein the fusion protein comprises an amino acid sequence having at least 85%, 90%, or 95% sequence identity to any of SEQ ID NOs. 55-61, 69-71, and 81.
25. A fusion protein, comprising:
- 15 a protein of interest to be delivered to a non-nuclear organelle;
- an organelle targeting sequence (OTS);
- a cell penetrating peptide (CPP); and
- a target enhancing sequence (TES);
- wherein the CPP is capable of interference with delivery of the protein of interest to the
20 non-nuclear organelle; and
- wherein the TES prevents said interference by the CPP.
26. The fusion protein of claim 25, wherein the CPP is located at the N-terminus of the fusion protein and wherein the TES is fused at the C-terminus of the CPP.
27. The fusion protein of claim 25 or 26, comprising, starting at the N-terminus:
- 25 CPP;

TES;

OTS; and

a protein of interest.

28. The fusion protein of claim 25, wherein the CPP is located at the C-terminus of the
5 fusion protein and wherein the TES is fused at the N-terminus of the CPP.

29. The fusion protein of any one of claims 25-28, wherein the non-nuclear organelle is selected from the group consisting of mitochondria, cytosol, lysosome, endoplasmic reticulum (ER), peroxisome and Golgi apparatus.

30. The fusion protein of claim 25 or 26, wherein the protein of interest in its naturally
10 occurring form is localized to mitochondria, cytosol, lysosome, endoplasmic reticulum (ER), peroxisome or Golgi apparatus.

31. The fusion protein of any one of claims 25-30, wherein the protein of interest is selected from the group consisting of Frataxin (FXN), Tafazin, Pyruvate Dehydrogenase (PDH), SLIRP, LRPPC, PARK2 protein, PARK6 protein, PARK7 protein, Phospholipase A2, Group VI and a
15 variant or derivative thereof.

32. The fusion protein of claim 31, wherein the protein of interest is Frataxin (FXN) or a variant or derivative thereof.

33. The fusion protein of claim 31, wherein the protein of interest is Pyruvate Dehydrogenase (PDH) or a variant or derivative thereof.

20 34. The fusion protein of any one of claims 25-33 wherein the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0.

35. The fusion protein of any one of claims 25-34, wherein the CPP comprises a peptide selected from the group consisting of HIV-TAT, galanin, mastoparan, transportan, penetratin, polyarginine, VP22, and a variant or derivative thereof.

25 36. The fusion protein of claim 35, wherein the CPP comprises HIV-TAT or a variant or derivative thereof.

37. The fusion protein of any one of claims 25-36, wherein the TES is a nuclear export signal peptide.
38. The fusion protein of claim 37, wherein the nuclear export signal peptide comprises a sequence having at least 85% sequence identity to any one of SEQ ID NOs. 36-43.
- 5 39. The fusion protein of claim 38, wherein the nuclear export signal peptide comprises a peptide selected from the group consisting of NES1, NES2, NES3, NES4, NES5, NES6, NES7, NES8, and a variant or derivative thereof.
40. The fusion protein of claim 39, wherein the nuclear export signal peptide comprises a peptide selected from the group consisting of NES1, NES2 and a variant or derivative thereof.
- 10 41. The fusion protein of any one of claims 25-36, wherein the TES is a protease sensitive peptide.
42. The fusion protein of claim 41, wherein the protease sensitive peptide comprises a ubiquitin-like modifier.
43. The fusion protein of claim 41, wherein the protease sensitive peptide comprises a
15 sequence having at least 85% sequence identity to any one of SEQ ID NOs. 18-31.
44. The fusion protein of claim 41, wherein the protease sensitive peptide comprises a peptide selected from the group consisting of ubiquitin, a caspase cleavage domain, a calpain cleavage domain, SUMO1, SUMO2, SUMO3, SUMO4, ISG15, Atg8, Atg12, NEDD8, and a variant or derivative thereof.
- 20 45. The fusion protein of any one of claims 25-44, further comprising a secretion signal (SS).
46. The fusion protein of any one of claims 25-45, further comprising an extracellular proteolytic site (EPS).
47. The fusion protein of any one of claims 25-46, wherein the fusion protein delivers the protein of interest to a non-nuclear organelle.
- 25 48. The fusion protein of any one claims 25-47, wherein the fusion protein delivers the protein of interest to mitochondria.

49. The fusion protein of claim 25, wherein the fusion protein comprises an amino acid sequence having at least 85%, 90% or 95% sequence identity to any of SEQ ID NOs. 55-61, 69-71 and 81.
50. A nucleic acid encoding the fusion protein of any one of claims 1-49.
- 5 51. An expression vector for introducing a fusion protein into a cell comprising the nucleic acid of claim 50.
52. The expression vector of claim 51, wherein the expression vector is selected from the group consisting of a retroviral vector, a DNA vector, a plasmid, an RNA vector, an adenoviral vector, an adenovirus associated vector, a lentiviral vector, a phagemid, a baculovirus and a
10 combination thereof.
53. A conjugate for intracellular delivery of proteins to non-nuclear organelles comprising the fusion protein of any one of claims 1-49 and a moiety linked to said fusion protein, wherein said moiety is selected from a group consisting of a radioactive label, a fluorescent label, a small molecule, and a polymeric molecule.
- 15 54. The conjugate of claim 53, wherein the polymeric molecule is polyethylene glycol (PEG).
55. A cell comprising the fusion protein of any one of claims 1-49, the nucleic acid of claim 50, the expression vector of any one of claims 51-52, the conjugate of any one of claims 53-54, or a combination thereof.
- 20 56. The cell of claim 55, wherein the cell is a stem cell or an iPS cell.
57. The cell of claim 55, wherein the cell is selected from the group consisting of a muscle progenitor cell, a neuron progenitor cell, a bone marrow stem cell, a bacterial cell line, or an yeast cell line.
58. A pharmaceutical composition comprising the fusion protein of any one of claims 1-49, a
25 conjugate of any one of claims 53-54, or a combination thereof and a pharmaceutically acceptable diluent, carrier, additive or excipient.

59. A method of delivery of a protein of interest to a cell, said method comprising contacting said cell with a fusion protein of any one of claims 1-49, the nucleic acid of claim 50, the vector of any one of claims 51-52 or the conjugate of any one of claims 53-54.
60. A method of intracellular delivery of a protein of interest to a non-nuclear organelle in a cell, said method comprising contacting said cell with a fusion protein of any one of claims 1-49, the nucleic acid of claim 50, the vector of any one of claims 51-52 or the conjugate of any one of claims 53-54.
61. The method of claim 59, wherein said non-nuclear organelle is mitochondria.
62. A therapeutic compound for treating a non-nuclear organelle associated disorder, said compound comprising the fusion protein of any one of claims 1-49, the nucleic acid of claim 50, the vector of any one of claims 51-52 or the conjugate of any one of claims 53-54.
63. The therapeutic compound of claim 62, wherein the non-nuclear organelle associated disorder is selected from the group consisting of Friedreich's ataxia (FDRA), Barth Syndrome, Parkinson's Disease, Wilson's Disease, Leigh Syndrome, Fibrosis, and PLA2G6-associated neurodegeneration (PLAN).
64. The therapeutic compound of claim 63, wherein the non-nuclear organelle associated disorder is FDRA.
65. The therapeutic compound of claim 63, wherein the non-nuclear organelle associated disorder is Parkinson's Disease.
66. A method for treating a non-nuclear organelle associated disorder, said method comprising administering to a subject in need thereof the fusion protein of any one of claims 1-49, the nucleic acid of claim 50, the vector of any one of claims 51-52, the conjugate of any one of claims 53-54 or the pharmaceutical composition of claim 58, such that said non-nuclear organelle associated disorder is treated.
67. The method of claim 66, wherein the non-nuclear organelle associated disorder is selected from the group consisting of Friedreich's ataxia (FDRA), Barth Syndrome, Parkinson's Disease, Wilson's Disease, Leigh Syndrome and Fibrosis.
68. The method of claim 67, wherein the non-nuclear organelle associated disorder is FDRA.

69. The method of claim 67, wherein the non-nuclear organelle associated disorder is Parkinson's Disease.

70. The method of any one of claims 66-69, wherein said subject is a human.

71. A method for increasing an amount of a protein of interest delivered to a cell, said
5 method comprising:

modifying a sequence of a fusion protein comprising the protein of interest and a cell penetrating peptide (CPP) by introducing into the fusion protein a target enhancing sequence (TES), thereby producing a modified fusion protein; and

contacting a cell with the modified fusion protein,

10 thereby increasing the amount of the protein of interest delivered to the cell relative to the amount of the protein of interest delivered to the cell by the fusion protein lacking the TES.

72. The method of claim 71, wherein in the modified fusion protein, the CPP is located at the N-terminus of the fusion protein and wherein the TES is fused at the C-terminus of the CPP.

73. The method of claim 71 or 72, wherein the modified fusion protein comprises, starting at
15 the N-terminus:

CPP;

TES; and

a protein of interest.

74. The method of claim 71 or 72, wherein the modified fusion protein comprises, starting at
20 the N-terminus:

CPP;

TES;

OTS; and

a protein of interest.

75. The method of claim 71, wherein in the modified fusion protein the CPP is located at the C-terminus of the fusion protein and wherein the TES is fused at the N-terminus of the CPP.
76. The method of claim 74, wherein the amount of the protein of interest delivered to an organelle targeted by the OTS is increased relative to the amount of the protein of interest delivered to the organelle by the fusion protein lacking the TES
77. The method of any one of claims 71-76, wherein the protein of interest is selected from the group consisting of Frataxin (FXN), Tafazin, Pyruvate Dehydrogenase (PDH), SLIRP, LRPPC, PARK2 protein, PARK6 protein, PARK7 protein, and Phospholipase A2, Group VI, or a variant or derivative thereof.
78. The method of claim 77, wherein the protein of interest is Frataxin (FXN) or a variant or derivative thereof.
79. The method of claim 77, wherein the protein of interest is PARK2 protein or a variant or derivative thereof.
80. The method of any one of claims 71-79, wherein the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0.
81. The method of any one of claims 71-79, wherein the CPP comprises a peptide selected from the group consisting of HIV-TAT, galanin, mastoparan, transportan, penetratin, polyarginine, VP22, and a variant or derivative thereof.
82. The method of claim 81, wherein the CPP comprises HIV-TAT or a variant or derivative thereof.
83. The method of any one of claims 71-82, wherein the TES is a nuclear export signal peptide.
84. The method of claim 83, wherein the nuclear export signal peptide comprises a sequence having at least 85% sequence identity to any one of SEQ ID NOs. 36-43.
85. The method of claim 84, wherein the nuclear export signal peptide comprises a peptide selected from the group consisting of NES1, NES2, NES3, NES4, NES5, NES6, NES7, NES8, and a variant or derivative thereof.

86. The method of claim 85, wherein the nuclear export signal peptide comprises a peptide selected from the group consisting of NES1, NES2 and a variant or derivative thereof.
87. The method of any one of claims 71-82, wherein the TES is a protease sensitive peptide.
88. The method of claim 87, wherein the protease sensitive peptide comprises a ubiquitin-
5 like modifier.
89. The method of claim 87, wherein the protease sensitive peptide comprises a sequence having at least 85% sequence identity to any one of SEQ ID NOs. 18-31.
90. The method of claim 87, the protease sensitive peptide comprises a peptide selected from the group consisting of ubiquitin, a caspase cleavage domain, a calpain cleavage domain,
10 SUMO1, SUMO2, SUMO3, SUMO4, ISG15, Atg8, Atg12, NEDD8, and a variant or derivative thereof.
91. The method of claim 71, wherein the modified fusion protein comprises an amino acid sequence having at least 85% sequence identity to any of SEQ ID NOs. 55-61, 69-71 and 81.