

SUMMARY

In one aspect of the present disclosure, there is provided a fusion protein, comprising:

a protein of interest to be delivered to a cell;

a cell penetrating peptide (CPP); and

a target enhancing sequence (TES).

In another aspect of the present disclosure, there is provided a nucleic acid encoding the fusion protein disclosed herein.

In a further aspect of the present disclosure, there is provided an expression vector for introducing a fusion protein into a cell comprising the nucleic acid of disclosed herein.

In another aspect of the present disclosure, there is provided a conjugate for intracellular delivery of proteins to non-nuclear organelles comprising the fusion protein disclosed herein 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.

In a further aspect of the present disclosure, there is provided a cell comprising the fusion protein disclosed herein, the nucleic acid disclosed herein, the expression vector disclosed herein, the conjugate disclosed herein, or a combination thereof.

In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising the fusion protein disclosed herein, a conjugate disclosed herein, or a combination thereof and a pharmaceutically acceptable diluent, carrier, additive or excipient.

In a further aspect of the present disclosure, there is provided a fusion protein disclosed herein, a nucleic acid disclosed herein, a vector disclosed herein or a conjugate disclosed herein, for delivery of a protein of interest to a cell.

In another aspect of the present disclosure, there is provided a fusion protein disclosed herein, a nucleic acid disclosed herein, a vector of disclosed herein or a conjugate disclosed herein, for delivery of a protein of interest to a non-nuclear organelle in a cell.

In a further aspect of the present disclosure, there is provided a use of the fusion protein disclosed herein, the nucleic acid disclosed herein, the vector disclosed herein, the conjugate disclosed herein or the pharmaceutical composition disclosed herein, in the manufacture of a medicament for treating a non-nuclear organelle associated disorder in a subject in need thereof.

In another aspect of the present disclosure, there is provided a modified fusion protein for increasing an amount of a protein of interest delivered to a cell, wherein:

the modified fusion protein is produced by 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); and

wherein a cell is to be contacted with the modified fusion protein, 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.

In a further aspect of the present disclosure, there is provided a modified fusion protein for use in increasing an amount of a protein of interest delivered to a cell, wherein:

the modified fusion protein is produced by 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); and

wherein a cell is to be contacted with the modified fusion protein, 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.

In a further aspect of the present disclosure, there is provided a fusion protein disclosed herein, a nucleic acid disclosed herein, a vector disclosed herein, a conjugate disclosed herein, or a pharmaceutical composition disclosed herein, for use in therapy.

The present disclosure provides fusion proteins that are capable of being efficiently delivered to cells, *e.g.*, in greater levels than previously achieved. The present disclosure further provides fusion proteins that are capable of efficiently delivering a therapeutic protein of interest to cells and achieving and maintaining the proper subcellular localization and/or function of the therapeutic protein of interest. For example, the present disclosure provides fusion proteins that

are capable of being efficiently delivered to non-nuclear organelles. An exemplary fusion protein provided by the present disclosure comprises, in addition to a protein of interest to be delivered to a cell, a cell penetrating peptide (CPP); and/or a target enhancing sequence (TES). In the fusion protein of the present disclosure, the CPP is capable of interference with delivery of the protein of interest to the protein's proper subcellular localization and/or with its function, and the TES prevents this interference by CPP. Another exemplary fusion protein provided by the present disclosure comprises, in addition to a protein of interest to be delivered to a non-nuclear organelle, an organelle targeting sequence (OTS); a cell penetrating peptide (CPP); and/or a target enhancing sequence (TES). In said fusion protein of the present disclosure, the CPP is capable of interference with delivery of the protein of interest to the non-nuclear organelle, and the TES prevents this interference by CPP.

The present disclosure also is based, at least on part, on the surprising discovery that including certain amino acid sequences (*i.e.*, TES) in a fusion protein that also comprises a protein of interest and a CPP, facilitated effective delivery of the protein of interest to the cell, *e.g.*, resulted in increased levels of the protein of interest in cells treated with the fusion protein, and allowed for proper subcellular localization and/or function of the protein of interest. In particular, the present inventors have discovered that cells treated with fusion proteins comprising a protein of interest, CPP and TES contained significantly higher amounts of the protein of interest than cells treated with a fusion protein comprising a protein of interest and CPP, but lacking TES. Thus, the present inventors have surprisingly discovered that introducing TES into a fusion protein comprising CPP and a protein of interest can significantly increase the amount of protein of interest in a cell that has been contacted with the fusion protein. The present inventors also surprisingly discovered that fusion proteins comprising a protein of interest, CPP and TES were correctly processed by the cellular machinery, achieved proper cellular localization, *e.g.*, were targeted to a non-nuclear organelle, and possessed the desired activity of the protein of interest when delivered to cells.

In some exemplary fusion proteins provided by the present disclosure, the TES comprises an amino acid sequence cleavable by an endogenous intracellular protease. In some examples, this TES is located immediately adjacent to the CPP, which, in turn, is located at the N-terminal end of the fusion protein. Upon entry of the fusion protein into the cell cytoplasm, the TES is cleaved by an intracellular nuclease. Without wishing to be bound by a specific theory, it is believed that cleavage of the TES facilitates removal of the CPP from the fusion protein and prevents the CPP from facilitating diffusion of the protein of interest across the plasma membrane and out of the cell. This allows the protein of interest to accumulate in the cell. The present disclosure is further based, at least on part, on the surprising discovery that including certain amino acid sequences (*i.e.*, TES) in a fusion protein that also comprises a protein of interest, a CPP and an OTS, facilitated effective delivery of the protein of interest to a non-nuclear organelle, such as mitochondria. In particular, the present inventors have discovered that fusion proteins comprising a protein of interest, CPP and an organelle targeting sequence (OTS), *e.g.* a mitochondrial targeting sequence (MTS), but not TES, localized to the cell nucleus instead of the mitochondria upon entry into the cell, due to the interference of the CPP with mitochondrial delivery of the protein of interest. The present inventors further discovered that including certain amino acid sequences, such as TES, in fusion proteins also comprising a protein of interest, a CPP and an OTS, *e.g.*, an MTS, facilitated delivery of the protein of interest to the mitochondria upon entry of the fusion protein into the cell.

In exemplary fusion proteins provided by the present disclosure, the TES comprises an amino acid sequence cleavable by an endogenous intracellular protease. In some examples, this TES is located immediately adjacent to the CPP, which, in turn, is located at the N-terminal end of the fusion protein. Upon entry of the fusion protein into the cell cytoplasm, the TES is cleaved by an intracellular nuclease. Cleavage of the TES facilitates removal of the CPP from the fusion protein and prevents interference of the CPP with delivery of the fusion protein to the mitochondria. In other exemplary fusion proteins of the present disclosure, the TES comprises a nuclear export signal peptide (NES), which prevents CPP-facilitated delivery of the fusion protein to the nucleus and, instead, facilitates delivery of the fusion protein to non-nuclear organelles, such as mitochondria.

In other exemplary fusion proteins of the present disclosure, the TES comprises a nuclear export signal peptide (NES), which prevents accumulation of the protein of interest in the nucleus.

In some examples, the present disclosure provides a fusion protein, comprising: a protein of interest to be delivered to a cell; a cell penetrating peptide (CPP); and a target enhancing sequence (TES).

In some examples, 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. In some examples, the fusion proteins comprises, or consists of, starting at the N-terminus: CPP; TES; and a protein of interest.

In some examples, 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.

In some examples, the fusion protein comprises, or consists of, starting at the C-terminus: a protein of interest; TES; and CPP.

In some examples, the protein of interest lacks an OTS.

In other examples, the fusion protein further comprises an organelle targeting sequence (OTS) exogenous to the protein of interest.

In some examples, the protein of interest comprises an OTS.

In some examples, the OTS is heterologous to the protein of interest. In some examples, the OTS is endogenous to the protein of interest.

In some examples, 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. In some examples, the protein of interest comprises Frataxin (FXN) or PARK2 protein (PARKIN), or a variant or derivative thereof.

In some examples, the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0. In some examples, 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. In some examples, the CPP comprises HIV-TAT or a variant or derivative thereof.

In some examples, the TES is a nuclear export signal peptide. In some examples, the nuclear export signal peptide comprises a sequence having at least 85% sequence identity to any one of SEQ ID NOs. 36-43. In some examples, 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. In some examples, the nuclear export signal peptide comprises a peptide selected from the group consisting of NES1 and NES2, or a variant or derivative thereof.

In some examples, the TES is a protease sensitive peptide. In some examples, the protease sensitive peptide comprises a ubiquitin-like modifier. In some examples, the protease sensitive peptide comprises a sequence having at least 85% sequence identity to any one of SEQ ID NOs. 18-31.

In some examples, 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.

In some examples, 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.

In some examples, the present disclosure provides a fusion protein, comprising: 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 non-nuclear organelle; and wherein the TES prevents said interference by the CPP.

In some examples, 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.

In some examples, the fusion protein comprises, or consists of, starting at the N-terminus: CPP; TES; OTS; and a protein of interest.

In some examples, 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. In some examples, the non-nuclear organelle is selected from the group consisting of mitochondria, cytosol, lysosome, endoplasmic reticulum (ER), peroxisome and Golgi apparatus. In some examples, the protein of interest in its naturally occurring form is localized to mitochondria, cytosol, lysosome, endoplasmic reticulum (ER), peroxisome or Golgi apparatus.

In some examples, 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. In some examples, the protein of interest is Frataxin (FXN) or a variant or derivative thereof. In some examples, the protein of interest is Pyruvate Dehydrogenase (PDH) or a variant or derivative thereof.

In some examples, the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0. In some examples, 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. In some examples, the CPP comprises HIV-TAT or a variant or derivative thereof.

In some examples, the TES is a nuclear export signal peptide. In some examples, the nuclear export signal peptide comprises a sequence having at least 85% sequence identity to any one of SEQ ID NOs. 36-43. In some examples, 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. In some examples, the nuclear export signal peptide comprises a peptide selected from the group consisting of NES1, NES2 and a variant or derivative thereof.

In some examples, the TES is a protease sensitive peptide. In some examples, the protease sensitive peptide comprises a ubiquitin-like modifier. In some examples, the protease sensitive peptide comprises a sequence having at least 85% sequence identity to any one of SEQ ID NOs. 18-31. In some examples, 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.

In some examples, the fusion protein of the present disclosure further comprises a secretion signal (SS).

In some embodiments, the fusion protein of the present disclosure further comprises an extracellular proteolytic site (EPS).

In some examples, the fusion protein delivers the protein of interest to a non-nuclear organelle. In some examples, the fusion protein delivers the protein of interest to mitochondria.

In some examples, 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.

In some examples, the present disclosure also provides nucleic acids encoding the fusion proteins of the present disclosure.

In some examples, the present disclosure also provides an expression vector for introducing a fusion protein into a cell comprising the nucleic acid of the disclosure. In some examples, 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 combination thereof.

In some examples, the present disclosure provides a conjugate for intracellular delivery of proteins to non-nuclear organelles comprising the fusion protein of the disclosure 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. In some examples, the polymeric molecule is polyethylene glycol (PEG).

In some examples, the present disclosure provides a cell comprising the fusion protein of the disclosure, the nucleic acid of the disclosure, the expression vector of the disclosure, the conjugate of the disclosure, or a combination thereof.

In some examples, the cell is a stem cell or an iPS cell. In some examples, 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 a yeast cell line.

In some examples, the present disclosure also provides a pharmaceutical composition comprising the fusion protein of the disclosure, a conjugate of the disclosure, or a combination thereof and a pharmaceutically acceptable diluent, carrier, additive or excipient.

In some examples, the present disclosure provides a method of delivery of a protein of interest to a cell, said method comprising contacting the cell with a fusion protein of the disclosure, the nucleic acid of the disclosure, the vector of the disclosure or the conjugate of the disclosure.

In some examples, the present disclosure also provides a method of intracellular delivery of a protein of interest to a non-nuclear organelle in a cell, the method comprising contacting said cell with a fusion protein of the disclosure, the nucleic acid of the disclosure, the vector of any one of the disclosure or the conjugate of the disclosure. In some examples, the non-nuclear organelle is mitochondria.

In some examples, the present disclosure also provides a therapeutic compound for treating a non-nuclear organelle associated disorder, the compound comprising the fusion protein the disclosure, the nucleic acid of the disclosure, the vector of the disclosure or the conjugate of the disclosure. In some examples, 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). In some examples, the non-nuclear organelle associated disorder is FDRA. In some examples, the non-nuclear organelle associated disorder is Parkinson's Disease.

In some examples, the present disclosure also provides a method for treating a non-nuclear organelle associated disorder, the method comprising administering to a subject in need thereof the fusion protein of the disclosure, the nucleic acid of the disclosure, the vector of the disclosure, the conjugate of the disclosure or the pharmaceutical composition of the disclosure, such that the non-nuclear organelle associated disorder is treated.

In some examples, 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. In some examples, the non-nuclear organelle associated disorder is FDRA. In some examples, the non-nuclear organelle associated disorder is Parkinson's Disease.

In some examples, the subject is a human.

In some examples, the present disclosure also provides a method for increasing an amount of a protein of interest delivered to a cell, the 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, 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.

In some examples, 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.

In some examples, the modified fusion protein comprises, starting at the N-terminus: CPP; TES; and a protein of interest.

In some examples, the modified fusion protein comprises, starting at the N-terminus: CPP; TES; OTS; and a protein of interest.

In some examples, 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. In some examples, 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.

In some examples, 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. In some examples, the protein of interest is Frataxin (FXN) or a variant or derivative thereof. In some examples, the protein of interest is PARK2 protein or a variant or derivative thereof.

In some examples, the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0. In some examples, 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. In some examples, the CPP comprises HIV-TAT or a variant or derivative thereof.

In some examples, the TES is a nuclear export signal peptide. In some examples, the nuclear export signal peptide comprises a sequence having at least 85% sequence identity to any one of SEQ ID NOs. 36-43. In some examples, 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. In some examples, the nuclear export signal peptide comprises a peptide selected from the group consisting of NES1, NES2 and a variant or derivative thereof.

In some examples, the TES is a protease sensitive peptide. In some examples, the protease sensitive peptide comprises a ubiquitin-like modifier. In some examples, the protease sensitive peptide comprises a sequence having at least 85% sequence identity to any one of SEQ ID NOs. 18-31. In some examples, 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.

In some examples, 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.

In one example, the present disclosure provides a fusion protein, comprising: 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 non-nuclear organelle; and wherein the TES prevents said interference by the CPP.

In some examples, the CPP is located at the N-terminus of the fusion protein and the TES is fused at the C-terminus of the CPP. In some examples, the present disclosure provides a fusion protein that comprises, starting at the N-terminus: CPP; TES; OTS; and a protein of interest.

In some examples, the CPP is located at the C-terminus of the fusion protein and the TES is fused at the N-terminus of the CPP.

In some examples, the non-nuclear organelle is selected from the group consisting of mitochondria, cytosol, lysosome, endoplasmic reticulum (ER), peroxisome and Golgi apparatus.

In some examples, the protein of interest in its naturally occurring form is localized to mitochondria, cytosol, lysosome, endoplasmic reticulum (ER), peroxisome or Golgi apparatus.

In some examples, the protein of interest is selected from the group consisting of Frataxin (FXN), Tafazin, Pyruvate Dehydrogenase (PDH), LRPPC, PARK2 protein, PARK6 protein, PARK7 protein, an A2 phospholipase Group VI, a transcription factor, and a variant or derivative thereof. In one example, the protein of interest is Frataxin (FXN) or a variant or derivative thereof. In one example, the protein of interest is pyruvate dehydrogenase (PDH) or a variant or derivative thereof.

In some examples, the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0. In further examples, 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. In some examples, the CPP comprises HIV-TAT or a variant or derivative thereof.

In some examples, the TES is a nuclear export signal peptide. In further examples, the nuclear export signal peptide comprises a sequence having at least 85% sequence identity to any one of SEQ ID NOs. 36-43. In some examples, 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. In some examples, the nuclear export signal peptide comprises a peptide selected from the group consisting of NES1, NES2 and a variant or derivative thereof.

In some examples, the TES is a protease sensitive peptide. In some examples, the protease sensitive peptide comprises a ubiquitin-like modifier. In some examples, the ubiquitin-like modifier comprises a sequence having at least 85% sequence identity to any one of SEQ ID NOs. 18-31.

In some examples, 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.

In some examples, the fusion protein provided by the present disclosure comprises a secretion signal (SS). In some examples, the fusion protein comprises an extracellular proteolytic site (EPS).

In some examples, the fusion protein delivers the protein of interest to a non-nuclear organelle. In a specific example, the fusion protein delivers the protein of interest to mitochondria.

In some examples, the fusion protein comprises a sequence having at least 85% sequence identity to any of SEQ ID NOs. 55-61.

In another example, the present disclosure provides a nucleic acid encoding the fusion protein of the disclosure.

In a related example, the present disclosure provides an expression vector comprising a nucleic acid of the disclosure for introducing a fusion protein into a cell .

In some examples, 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 combination thereof.

In another example, the present disclosure provides a protein conjugate for intracellular delivery of proteins to non-nuclear organelles that comprises a fusion protein of the disclosure and a moiety linked to the fusion protein, wherein the moiety is selected from the group consisting of a radioactive label, a fluorescent label, a small molecule, and a polymeric molecule. In one example, the polymeric molecule is polyethylene glycol (PEG).

In another example, the present disclosure provides a cell comprising a fusion protein, a nucleic acid, an expression vector or a conjugate of the disclosure, or a combination thereof. The cell may be a transformed cell, *e.g.*, a cell which has been transformed with a fusion protein, a nucleic acid, an expression vector or a conjugate of the disclosure, or a combination thereof. In some examples, the cell is stem cell or an iPS cell. In some examples, a cell may be selected from the group consisting of a muscle progenitor cell, a neuron progenitor cell, a bone marrow stem cell, a bacterial cell, or a yeast cell.

In another example, the present disclosure provides a pharmaceutical composition comprising a fusion protein or a protein conjugate of the disclosure, or a combination thereof, and a pharmaceutically acceptable diluent, carrier, additive or excipient.

In another example, the present disclosure provides a method of intracellular delivery of a protein of interest to a non-nuclear organelle in a cell, comprising contacting the cell with a fusion protein, a nucleic acid, a vector or a protein conjugate of the disclosure. In one example, the non-nuclear organelle is mitochondria.

In another example, the present disclosure provides a therapeutic compound for treating a non-nuclear organelle associated disorder, which comprises a fusion protein, a nucleic acid, a vector or a protein conjugate of the disclosure. In some, 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). In one example, PLAN may comprise a disorder selected from the group consisting of infantile neuroaxonal dystrophy (INAD), atypical neuroaxonal dystrophy (ANAD), Parkinsonian Syndrome which contains adult onset dystonia parkinsonism (DP) and autosomal recessive early-onset parkinsonism (AREP). In one example, the non-nuclear organelle associated disorder is FDRA.

In a related example, the present disclosure provides a method for treating a non-nuclear organelle associated disorder, comprising administering to a subject in need thereof a fusion protein, a nucleic acid, a vector, a protein conjugate, or a pharmaceutical composition of the disclosure, such that the non-nuclear organelle associated disorder is treated. In some examples, 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. In one specific example, the non-nuclear organelle associated disorder is FDRA.

In some examples, the subject is a human.

This Summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This Summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used to limit the scope of the claimed subject matter.

CLAIMS

1. A fusion protein, comprising:

a protein of interest to be delivered to a cell;

a cell penetrating peptide (CPP); and

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; or 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.
3. The fusion protein of claim 1 or 2, comprising or consisting of, starting at the N-terminus:

CPP;

TES; and

a protein of interest; or

comprising or consisting of, starting at the C-terminus:

a protein of interest;

TES; and

CPP.
4. The fusion protein of any one of claims 1-3, wherein the fusion protein lacks an organelle targeting sequence (OTS); or wherein the fusion protein further comprises an organelle targeting sequence (OTS).
5. The fusion protein of claim 4, wherein the OTS is heterologous to the protein of interest; or wherein the OTS is endogenous to the protein of interest.

6. The fusion protein of claim 1, 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.
7. The fusion protein of claim 11, wherein the protein of interest comprises Frataxin (FXN) or PARK2 protein, or a variant or derivative thereof.
8. The fusion protein of any one of claims 1-7, wherein the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0; or 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.
9. The fusion protein of claim 8, wherein the CPP comprises HIV-TAT or a variant or derivative thereof.
10. The fusion protein of any one of claims 1-9, wherein the TES is a nuclear export signal peptide.
11. The fusion protein of claim 10, wherein the nuclear export signal peptide comprises a sequence having at least 85% sequence identity to any one of SEQ ID NOs. 36-43.
12. The fusion protein of claim 11, wherein the nuclear export signal peptide comprises a peptide selected from the group consisting of NES1 and NES2, or a variant or derivative thereof.
13. The fusion protein of any one of claims 1-12, wherein the TES is a protease sensitive peptide.
14. The fusion protein of claim 13, wherein the protease sensitive peptide comprises a ubiquitin-like modifier.
15. The fusion protein of claim 14, wherein the protease sensitive peptide comprises a sequence having at least 85% sequence identity to any one of SEQ ID NOs. 18-31.
16. The fusion protein of claim 13, 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.

17. 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.

18. The fusion protein of claim 1, comprising:

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 non-nuclear organelle; and

wherein the TES prevents said interference by the CPP.

19. The fusion protein of claim 18, 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; or 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.

20. The fusion protein of claim 19, comprising, starting at the N-terminus:

CPP;

TES;

OTS; and

a protein of interest.

21. The fusion protein of claim 18 or 19, wherein the non-nuclear organelle is selected from the group consisting of mitochondria, cytosol, lysosome, endoplasmic reticulum (ER), peroxisome and Golgi apparatus.

22. The fusion protein of claim 18, wherein the protein of interest in its naturally occurring form is localized to mitochondria, cytosol, lysosome, endoplasmic reticulum (ER), peroxisome or Golgi apparatus; or 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.
23. The fusion protein of claim 22, wherein the protein of interest is Frataxin (FXN) or a variant or derivative thereof or wherein the protein of interest is Pyruvate Dehydrogenase (PDH) or a variant or derivative thereof.
24. The fusion protein of any one of claims 18-23, further comprising a secretion signal (SS) and/or an extracellular proteolytic site (EPS).
25. The fusion protein of claim 18, 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.
26. A nucleic acid encoding the fusion protein of any one of claims 1-25.
27. An expression vector for introducing a fusion protein into a cell comprising the nucleic acid of claim 26.
28. A conjugate for intracellular delivery of proteins to non-nuclear organelles comprising the fusion protein of any one of claims 1-25 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.
29. A cell comprising the fusion protein of any one of claims 1-25, the nucleic acid of claim 26, the expression vector of claim 27, the conjugate of claim 28, or a combination thereof.
30. A pharmaceutical composition comprising the fusion protein of any one of claims 1-25, a conjugate of claim 28, or a combination thereof and a pharmaceutically acceptable diluent, carrier, additive or excipient.
31. The fusion protein of any one of claims 1-25, the nucleic acid of claim 26, the vector of claim 27 or the conjugate of claim 28, for delivery of a protein of interest to a cell.

32. The fusion protein of any one of claims 1-25, the nucleic acid of claim 26, the vector of claim 27 or the conjugate of claim 28, for delivery of a protein of interest to a non-nuclear organelle in a cell.

33. The fusion protein, nucleic acid, vector or conjugate of claim 32, wherein said non-nuclear organelle is mitochondria.

34. Use of the fusion protein of any one of claims 1-25, the nucleic acid of claim 26, the vector of claim 27, the conjugate of claim 28 or the pharmaceutical composition of claim 30, in the manufacture of a medicament for treating a non-nuclear organelle associated disorder in a subject in need thereof.

35. The use of claim 34, 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.

36. The use of claim 35, wherein the non-nuclear organelle associated disorder is FDRA or Parkinson's Disease.

37. The use of any one of claims 34-36, wherein said subject is a human.

38. A modified fusion protein for increasing an amount of a protein of interest delivered to a cell, wherein:

the modified fusion protein is produced by 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); and

wherein a cell is to be contacted with the modified fusion protein,

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.

39. A modified fusion protein for use in increasing an amount of a protein of interest delivered to a cell, wherein:

the modified fusion protein is produced by 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); and

wherein a cell is to be contacted with the modified fusion protein,

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.

40. The fusion protein of any one of claims 1-25, the nucleic acid of claim 26, the vector of claim 27, the conjugate of claim 28, or the pharmaceutical composition of claim 30, for use in therapy.