

Intellectual Disability, Partial Epilepsy and Episodic Ataxia, Febrile Seizures, Autosomal Dominant Partial Epilepsy with Auditory Features (ADPEAF), PNPO-Deficiency, Progressive Myoclonus Epilepsy, Action Myoclonus – Renal Failure (AMRF), CDKL5 deficiency disorder, and Benign Familial Infantile Seizures (BFIS).

- 5 **[0006]** By way of example, Dravet Syndrome is a rare and catastrophic form of intractable epilepsy that begins in infancy. Initially, patients experience prolonged seizures. In their second year, additional types of seizure begin to occur, which typically coincide with a developmental decline, possibly due to repeated cerebral hypoxia. This leads to poor development of language and motor skills. Mutations in SCN1A (encoding the voltage-gated sodium channel α subunit
- 10 Nav1.1), SCN1B (encoding the voltage-gated sodium channel β 1 subunit), SCN2A (encoding Nav1.2), SCN3A (encoding Nav1.3), SCN9A (encoding Nav1.7), GABRG2 (encoding the γ -aminobutyric acid receptor γ 2 subunit), GABRD (encoding the γ -aminobutyric acid receptor Δ subunit) and/or PCDH19 (encoding Protocadherin-19) genes have been linked to Dravet Syndrome.
- 15 **[0007]** Dravet syndrome may be caused by a nonsense mutation in, for example, the SCN1A gene, resulting in a premature termination codon and a lack of or reduced amount of untruncated or functional protein. The SCN1A gene normally codes for the neuronal voltage-gated sodium channel α subunit, Na(V)1.1. In mouse models, loss-of-function mutations in SCN1A have been observed to result in a decrease in sodium currents and impaired excitability of GABAergic
- 20 interneurons of the hippocampus.
- [0008]** Despite the efforts made to date, there is a need in the art for improved compositions and methods for treating disorders mediated by premature termination codons, such as Dravet syndrome.

SUMMARY OF THE INVENTION

- 25 **[0009]** The disclosure is based, in part, upon the discovery that is possible to express multiple (*e.g.*, two or three) suppressor tRNAs using a single expression vector. Each suppressor tRNA permits an amino acid to be incorporated into a gene product encoded by a gene in a mammalian cell at a position that would otherwise result in a truncated gene product caused by a premature termination codon (PTC) in the gene. Expression of multiple suppressor tRNAs from a single
- 30 expression vector allows for the single expression vector to treat a disease mediated by multiple, different PTCs in the same subject and/or treat a disease mediated by multiple, different PTCs in multiple, different subjects. The disclosure is further based, in part, upon the discovery of

optimal combinations of suppressor tRNAs that allow for treatment of the greatest possible patient populations.

[0010] In one aspect, the present disclosure provides an expression vector comprising: (a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that

5 hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid; b) a second nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being

aminoacylated with a second amino acid; and (c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon,

10 and is capable of being aminoacylated with a third amino acid. In another aspect, the present disclosure provides an expression vector comprising: (a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop

codon, and is capable of being aminoacylated with arginine; (b) a second nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG

15 premature stop codon, and is capable of being aminoacylated with a glutamine; and (c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with

glutamine, wherein the expression vector comprises in a 5' to 3' orientation, the third nucleotide sequence, the first nucleotide sequence, and the second nucleotide sequence. Accordingly, in one

20 example, the disclosure provides an expression vector comprising:(a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a

second nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being

25 aminoacylated with a second amino acid; and optionally, (c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a third

premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid.

[0011] In certain examples, the first amino acid is selected from arginine, tryptophan, cysteine,

30 serine, glycine, and leucine (*e.g.*, the first amino acid is arginine). In certain examples, the second amino acid is selected from glutamine, glutamic acid, tyrosine, tryptophan, lysine, serine, and leucine (*e.g.*, the second amino acid is glutamine). In certain examples, the third amino acid is selected from glutamine, glutamic acid, tyrosine, lysine, serine, and leucine. In certain

examples, the second and third amino acid are the same, for example, the second and third amino acid are selected from glutamine, glutamic acid, tyrosine, lysine, serine, and leucine.

[0012] In certain examples: (i) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is lysine; (ii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamic acid; (iii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is tyrosine; (iv) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is leucine; (v) the first amino acid is arginine, the second amino acid is tryptophan, and the third amino acid is glutamic acid; or (vi) the first amino acid is arginine, the second amino acid is tyrosine, and the third amino acid is glutamic acid. In certain examples: (i) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamine; (ii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamic acid; (iii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is lysine; (iv) the first amino acid is arginine, the second amino acid is tryptophan, and the third amino acid is glutamine; or (v) the first amino acid is arginine, the second amino acid is glutamic acid, and the third amino acid is glutamine. In certain examples: (i) the first amino acid is

arginine, the second amino acid is glutamine, and the third amino acid is glutamine; (ii) the first amino acid is tryptophan, the second amino acid is glutamic acid, and the third amino acid is glutamic acid; (iii) the first amino acid is cysteine, the second amino acid is tyrosine, and the third amino acid is tyrosine; (iv) the first amino acid is serine, the second amino acid is lysine, and the third amino acid is lysine; (v) the first amino acid is glycine, the second amino acid is serine, and the third amino acid is serine; or (vi) the first amino acid is leucine, the second amino acid is leucine, and the third amino acid is leucine.

[0013] In certain examples, the first, second, and/or third suppressor tRNA comprises a nucleotide sequence set forth in **TABLE 2** or **TABLE 3**. For example, (i) when the first amino acid is arginine, the first suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-22, and 35, (ii) when the second amino acid is glutamine, the second suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 178-182, 186, and 187, and/or (iii) when the third amino acid is glutamine, the third suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45.

[0014] In certain examples, the expression vector comprises 1, 2, 3, 4, or more than 4 copy numbers of the nucleotide sequence encoding the first, second, and/or third suppressor tRNA.

[0015] In certain examples, the expression vector comprises a nucleotide sequence corresponding to a genomic DNA sequence flanking a wild-type tRNA gene. For example, in certain examples, the expression vector comprises a nucleotide sequence set forth in **TABLE 4**.

In certain examples, the nucleotide sequence set forth in **TABLE 4** is selected from SEQ ID NOs: 869-888. In certain examples, the nucleotide sequence set forth in **TABLE 4** is operably linked to the nucleotide sequence encoding the first, second, and/or third suppressor tRNA. In certain examples, in the expression vector, the nucleotide sequence set forth in **TABLE 4** is 5' to the nucleotide sequence encoding the first, second, and/or third suppressor tRNA. In certain examples, in the expression vector, the nucleotide sequence set forth in **TABLE 4** is immediately 5' to (i.e., adjacent) the nucleotide sequence encoding the first, second, and/or third suppressor tRNA.

[0016] In certain examples, the expression vector is a viral vector, *e.g.*, a DNA virus vector, *e.g.*, an adeno-associated virus (AAV) vector.

[0017] In another aspect, the disclosure provides a pharmaceutical composition comprising any of the foregoing expression vectors and a pharmaceutically acceptable excipient.

[0018] In another example, the disclosure provides a pharmaceutical composition comprising: (a) a first suppressor tRNA that comprises an anticodon that hybridizes to a first premature stop codon

(*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second suppressor tRNA that comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid.

[0019] In certain examples, the first, second, and/or third suppressor tRNA comprises a nucleotide sequence set forth in **TABLE 2** or **TABLE 3**. For example, (i) when the first amino acid is arginine, the first suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-22, and 35, (ii) when the second amino acid is glutamine, the second suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 178-182, 186, and 187, and/or (iii) when the third amino acid is glutamine, the third suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45.

[0020] In certain examples, the first, second, and/or third suppressor tRNA comprises one or more naturally occurring nucleotide modifications, *e.g.*, selected from 5-methyl uridine, 5-carbamoylmethyluridine, 5-carbamoyl-methyl-2-O-methyluridine, 5-methoxycarbonylmethyluridine, 5-methoxycarbonylmethyl-2-thiouridine, pseudouridine, dihydrouridine, 1-methyladenosine, and inosine. In certain examples, the tRNA is not conjugated to, or associated with, another moiety, *e.g.*, a carrier particle, *e.g.*, an aminolipid particle. In certain examples, the composition does not comprise a nanoparticle and/or an aminolipid delivery compound.

[0021] In yet another aspect, the present disclosure provides an *in vitro* method of expressing in a mammalian cell a functional gene product encoded by a gene containing a premature termination codon, the method comprising contacting the cell with an effective amount of the expression vector as described herein or the pharmaceutical composition as described herein, thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon. In another example, the disclosure provides a method of expressing in a mammalian cell a functional gene product encoded by a gene containing a premature termination codon, the method comprising contacting the cell with an effective amount of any of the foregoing expression vectors or pharmaceutical compositions, thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon.

[0022] In another example, the disclosure provides a method of expressing in a mammalian cell a functional gene product encoded by a gene containing a first, second, and/or third premature termination codon, the method comprising contacting the cell with effective amount of: (a) a first expression vector comprising a nucleotide sequence encoding a first suppressor tRNA that
5 comprises an anticodon that hybridizes to a first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second expression vector comprising a nucleotide sequence encoding a second suppressor tRNA that comprises an

anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third expression vector comprising a nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid, thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon.

[0023] In another example, the disclosure provides a method of expressing in a mammalian cell a functional gene product encoded by a gene containing a first, second, and/or third premature termination codon, the method comprising contacting the cell with effective amount of: (a) a first suppressor tRNA that comprises an anticodon that hybridizes to a first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second suppressor tRNA that comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid, thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon.

[0024] In certain examples of any of the foregoing methods, the first, second, and/or third suppressor tRNA comprises a nucleotide sequence set forth in **TABLE 2** or **TABLE 3**. For example, (i) when the first amino acid is arginine, the first suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-22, and 35, (ii) when the second amino acid is glutamine, the second suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 178-182, 186, and 187, and/or (iii) when the third amino acid is glutamine, the third suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45.

[0025] In certain examples of any of the foregoing methods, the gene is a gene set forth in **TABLE 5** or **TABLE 6**. In certain examples, the gene is an SCN1A or dystrophin gene.

[0026] In certain examples of any of the foregoing methods, the cell is a human cell. In certain examples, the cell is a central nervous system cell, *e.g.*, a neuron. In certain examples, the tRNA becomes aminoacylated in the cell.

[0027] In a further aspect, the present disclosure provides a use of the expression vector as described herein or the pharmaceutical composition as described herein in the manufacture of a

medicament for the treatment of a premature termination codon-mediated disorder in a subject in need thereof, wherein the subject has a gene with a premature termination codon. In another example, the disclosure provides a method of treating a premature termination codon-mediated disorder in a subject (or a population of subjects) in need thereof, wherein the

subject(s) have a gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject(s) an effective amount of any of the foregoing expression vectors or any of the foregoing pharmaceutical compositions, thereby to treat the disorder in the subject.

5 **[0028]** In another example, the disclosure provides a method of treating a premature termination codon-mediated disorder in a subject (or a population of subjects) in need thereof wherein the subject(s) have a gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject(s) an effective amount of: (a) a first expression vector comprising a nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon
10 that hybridizes to a first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second expression vector comprising a nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third expression vector comprising a nucleotide sequence
15 encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid; thereby to treat the disorder in the subject(s).

[0029] In a further aspect, the present disclosure provides a use of an expression vector comprising: (a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an
20 anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid; (b) a second nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid; and (c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop
25 codon, and is capable of being aminoacylated with a third amino acid, wherein the expression vector comprises in a 5' to 3' orientation, the third nucleotide sequence, the first nucleotide sequence, and the second nucleotide sequence, in the manufacture of a medicament for the treatment of a premature termination codon-mediated disorder in a subject in need thereof, wherein the subject has a gene with a first, second, and/or third premature termination codon. In
30 another example, the disclosure provides a method of treating a premature termination codon-mediated disorder in a subject (or a population of subjects) in need thereof wherein the subject(s) have a gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject(s) an effective amount of: (a) a first suppressor tRNA that comprises an anticodon that hybridizes to a first premature stop codon (*e.g.*, TGA), and is

capable of being aminoacylated with a first amino acid; (b) a second suppressor tRNA that comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop codon (*e.g.*, TAA),
5 and is capable of being aminoacylated with a third amino acid; thereby to treat the disorder in the subject(s).

[0030] In certain examples of any of the foregoing methods, the first, second, and/or third suppressor tRNA comprises a nucleotide sequence set forth in **TABLE 2** or **TABLE 3**. For example, (i) when the first amino acid is arginine, the first suppressor tRNA may comprise a
10 nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-22, and 35, (ii) when the second amino acid is glutamine, the second suppressor tRNA may comprise a nucleotide sequence

selected from SEQ ID NOs: 178-182, 186, and 187, and/or (iii) when the third amino acid is glutamine, the third suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45.

[0031] In certain examples of any of the foregoing methods, the disorder is a disorder set forth in

TABLE 5 or **TABLE 6**. In certain examples, the disorder is Dravet Syndrome or Duchenne Muscular Dystrophy.

[0032] In another example, the disclosure provides a method of treating Dravet Syndrome in a subject (or a population of subjects) in need thereof wherein the subject(s) have an SCN1A gene with a first, second, and/or third premature termination codon, the method comprising

administering to the subject an effective amount of an expression vector comprising: (a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to the first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to the second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to the third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid; thereby to treat Dravet Syndrome in the subject(s). In certain examples, (i) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is lysine; (ii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamic acid; (iii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is tyrosine; (iv) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is leucine; (v) the first amino acid is arginine, the second amino acid is tryptophan, and the third amino acid is glutamic acid; or (vi) the first amino acid is arginine, the second amino acid is tyrosine, and the third amino acid is glutamic acid.

[0033] In another example, the disclosure provides a method of treating Duchenne Muscular Dystrophy in a subject (or a population of subjects) in need thereof wherein the subject(s) have a dystrophin gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject(s) an effective amount of an expression vector comprising: (a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to the first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second nucleotide sequence encoding a second

suppressor tRNA that comprises an anticodon that hybridizes to the second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to the third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid; thereby to treat Duchenne Muscular Dystrophy in the subject(s). In certain examples: (i) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamine; (ii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamic acid; (iii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is lysine; (iv) the first amino acid is arginine, the second amino acid is tryptophan, and the third amino acid is glutamine; or (v) the first amino acid is arginine, the second amino acid is glutamic acid, and the third amino acid is glutamine.

[0034] These and other examples and features of the disclosure are described in the following detailed description and claims.

DESCRIPTION OF THE DRAWINGS

[0035] The invention can be more completely understood with reference to the following drawings.

[0036] **FIGURE 1** is a schematic representation of a transcript (*e.g.*, an SCN1A transcript) containing a premature termination codon (PTC) which leads to a truncated protein product (*e.g.*, a protein product in a subject with Dravet syndrome). Native termination codons are indicated as shaded circles, and premature termination codons are indicated as unshaded circles. Expression of a suppressor tRNA (*e.g.*, an anticodon modified arginine tRNA) charged with its cognate amino acid (A.A.) allows read-through of the PTC and facilitates expression of the full-length protein.

[0037] **FIGURE 2A** is a consensus tRNA secondary structure. The numbering of the residues is based on the tRNA numbering system described in Steinberg *et al.* (1993) NUCLEIC ACIDS RES. 21:3011-15. **FIGURE 2B** is a table showing the modification profile for tRNA sequences from the cytosol of certain eukaryotic organisms. The ratios in the table indicate the frequency of occurrence of listed nucleotide at the numbered position shown in **FIGURE 2A**. The abbreviations for the modified residues are defined in Motorin *et al.* (2005) "Transfer RNA Modification," *ENCYCLOPEDIA OF LIFE SCIENCES*, John Wiley & Sons, Inc.

WHAT IS CLAIMED IS:

1. An expression vector comprising:
 - (a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid;
 - (b) a second nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid; and
 - (c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with a third amino acid.
2. The expression vector of claim 1, wherein the expression vector comprises in a 5' to 3' orientation, the third nucleotide sequence, the first nucleotide sequence, and the second nucleotide sequence.
3. The expression vector of claim 1 or 2, wherein:
 - (a) the first amino acid:
 - (i) is selected from arginine, tryptophan, cysteine, serine, glycine, and leucine; and/or
 - (ii) is arginine;
 - (b) the second amino acid:
 - (i) is selected from glutamine, glutamic acid, tyrosine, tryptophan, lysine, serine, and leucine; and/or
 - (ii) is glutamine;
 - (c) the third amino acid:
 - (i) is selected from glutamine, glutamic acid, tyrosine, lysine, serine, and leucine; and/or
 - (ii) is glutamine;
 - (d) the second and third amino acid are the same; and/or

- (e) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamine.
4. The expression vector of any one of claims 1-3, wherein:
- (a) the first, second, and/or third suppressor tRNA comprises a nucleotide sequence set forth in **TABLE 2** or **TABLE 3**;
 - (b) the first amino acid is arginine and the first suppressor tRNA comprises a nucleotide sequence selected from any one of SEQ ID NOs: 18, 22, 6, 8, 9, 35, 17, 21, 20, 16, 19, 7, 11, 1, 2, 387, and 388;
 - (c) the second amino acid is glutamine and the second suppressor tRNA comprises a nucleotide sequence selected from any one of SEQ ID NOs: 181, 178, 187, 180, 179, 186, and 182;
 - (d) the third amino acid is glutamine and the third suppressor tRNA comprises a nucleotide sequence selected from any one of SEQ ID NOs: 451, 457, 447, 39, 36, 45, 38, 37, 44, and 40.
5. An expression vector comprising:
- (a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with arginine;
 - (b) a second nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a glutamine; and
 - (c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with glutamine,
- wherein the expression vector comprises in a 5' to 3' orientation, the third nucleotide sequence, the first nucleotide sequence, and the second nucleotide sequence.
6. The expression vector of claim 5, wherein:
- (a) the first suppressor tRNA comprises a nucleotide sequence selected from any one of

SEQ ID NOs: 18, 22, 6, 8, 9, 35, 17, 21, 20, 16, 19, 7, 11, 1, 2, 387, and 388;

(b) the second suppressor tRNA comprises a nucleotide sequence selected from any one of SEQ ID NOs: 181, 178, 187, 180, 179, 186, and 182; and/or

(c) third suppressor tRNA comprises a nucleotide sequence selected from any one of SEQ ID NOs: 451, 457, 447, 39, 36, 45, 38, 37, 44, and 40.

7. The expression vector of any one of claims 1-6, wherein the expression vector comprises 1, 2, 3, 4, or more than 4 copy numbers of the nucleotide sequence encoding the first, second, and/or third suppressor tRNA.
8. The expression vector of any one of claims 1-7, wherein:
 - (a) the expression vector further comprises a nucleotide sequence set forth in **TABLE 4**;
 - (b) the expression vector comprises a nucleotide sequence selected from any one of SEQ ID NOs: 869-888;
 - (c) the first nucleotide sequence, the second nucleotide sequence, and the third nucleotide sequence are each operably linked to a nucleotide sequence set forth in **TABLE 4**; and/or
 - (d) the first nucleotide sequence, the second nucleotide sequence, and the third nucleotide sequence are each operably linked to a nucleotide sequence selected from any one of SEQ ID NOs: 869-888.
9. The expression vector of any one of claims 1-8, wherein the expression vector is a viral vector, optionally wherein the viral vector is a DNA virus vector and/or an adeno-associated virus (AAV) vector.
10. A pharmaceutical composition comprising the expression vector of any one of claims 1-9 and a pharmaceutically acceptable excipient.
11. The pharmaceutical composition of claim 10, wherein the first, second, and/or third suppressor tRNA comprises a naturally occurring nucleotide modification and/or the first, second, and/or third suppressor tRNA comprises one or more nucleotide modifications selected from 5-methyl uridine, 5-carbamoylmethyluridine, 5-carbamoyl-methyl-2-O-methyluridine, 5-methoxy-carbonylmethyluridine, 5-methoxycarbonylmethyl-2-thiouridine, pseudouridine, dihydrouridine, 1-methyladenosine, and inosine.

12. The pharmaceutical composition of claim 10 or 11, wherein:
 - (a) the expression vector or tRNA is not conjugated to or associated with another moiety or a carrier particle: and/or
 - (b) the composition does not comprise an aminolipid particle, an aminolipid delivery compound, or a nanoparticle.
13. An *in vitro* method of expressing in a mammalian cell a functional gene product encoded by a gene containing a premature termination codon, the method comprising contacting the cell with an effective amount of the expression vector of any one of claims 1-9 or the pharmaceutical composition of any one of claims 10-12, thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon.
14. The method of claim 13, wherein the gene:
 - (a) is a gene set forth in **TABLE 5** or **TABLE 6**;
 - (b) is a gene set forth in **TABLE 5**; and/or
 - (c) is SCN1A or dystrophin..
15. The method of claim 13 or 14, wherein:
 - (a) the cell is a human cell; and/or
 - (b) the tRNA becomes aminoacylated in the cell.
16. Use of the expression vector of any one of claims 1-9 or the pharmaceutical composition of any one of claims 10-12 in the manufacture of a medicament for the treatment of a premature termination codon-mediated disorder in a subject in need thereof, wherein the subject has a gene with a premature termination codon.
17. Use of an expression vector comprising:
 - (a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid;
 - (b) a second nucleotide sequence encoding a second suppressor tRNA that comprises an

anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid; and

(c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with a third amino acid,

wherein the expression vector comprises in a 5' to 3' orientation, the third nucleotide sequence, the first nucleotide sequence, and the second nucleotide sequence;

in the manufacture of a medicament for the treatment of a premature termination codon-mediated disorder in a subject in need thereof, wherein the subject has a gene with a first, second, and/or third premature termination codon.

18. The use of claim 16 or 17, wherein:

(a) the first, second, and/or third suppressor tRNA comprises a nucleotide sequence set forth in **TABLE 2** or **TABLE 3**;

(b) the first amino acid is arginine and the first suppressor tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 18, 22, 6, 8, 9, 35, 17, 21, 20, 16, 19, 7, 11, 1, 2, 387, and 388;

(c) the second amino acid is glutamine and the second suppressor tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 181, 178, 187, 180, 179, 186, and 182; and/or

(d) the third amino acid is glutamine and the third suppressor tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 451, 457, 447, 39, 36, 45, 38, 37, 44, and 40.

19. The use of any one of claims 16-18, wherein the disorder:

(a) is a disorder set forth in **TABLE 5** or **TABLE 6**;

(b) is a disorder set forth in **TABLE 5**; and/or

(c) is Dravet Syndrome or Duchenne Muscular Dystrophy.

20. The use of any one of claims 16-19, wherein the subject is a human.