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(54) Title: METHODS AND COMPOSITIONS FOR TREATING A PREMATURE TERMINATION CODON-MEDIATED DISORDER

(57) Abstract: The invention relates generally to modified tRNAs and the use of modified tRNAs to express in a mammalian cell a functional gene product encoded by a gene containing a premature termination codon and/or to treat a disease mediated by a premature termination codon, e.g., Dravet syndrome.



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**METHODS AND COMPOSITIONS FOR TREATING
A PREMATURE TERMINATION CODON-MEDIATED DISORDER**

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application
5 No. 62/929,428, filed November 1, 2019, which is incorporated herein by reference in its
entirety for all purposes.

FIELD OF THE INVENTION

[0002] The invention relates generally to methods and compositions for expressing a gene
product encoded by a gene containing a premature termination codon and/or treating a disorder
10 mediated by a premature termination codon.

BACKGROUND

[0003] Protein synthesis is directed by a genetic code that includes 61 three-base-pair codons
encoding amino acids that are incorporated into the protein being synthesized and 3 three-base-
pair codons (referred to as stop or termination codons) that terminate the synthesis of a protein.
15 When a nucleic acid sequence encoding a protein is mutated to contain a premature termination
codon rather than a codon for the next amino acid, the resulting protein is prematurely
terminated, which is often nonfunctional or less functional than the untruncated or full length
protein. Such mutations, termed nonsense mutations, are often associated with, or are a
causative agent in numerous different genetic diseases.

20 [0004] A number of disorders are associated with, or are caused by nonsense mutations. These
include epilepsies, for example, Dravet Syndrome, Genetic Epilepsy with Febrile Seizures
(GEFS), Benign Familial Infantile Epilepsy (BFIE), Early Infantile Epileptic Encephalopathy
(EIEE), Lennox-Gastaut Syndrome, Rett Syndrome, PPM-X Syndrome, Ohtahara Syndrome,
Episodic Ataxia, Hemiplegic Migraine, Idiopathic Generalized Epilepsy, FOXP1 Syndrome,
25 Familial Focal Epilepsy with Variable Foci (FFEVF), Childhood-Onset Epileptic
Encephalopathy, SYNGAP1-Related Intellectual Disability, Pyridoxine-Dependent Epilepsy,
Familial Infantile Myoclonic Epilepsy (FIME), Myoclonic Astatic Epilepsy, X-Linked
Intellectual Disability, Partial Epilepsy and Episodic Ataxia, Febrile Seizures, Autosomal
Dominant Partial Epilepsy with Auditory Features (ADPEAF), PNPO-Deficiency, Progressive
30 Myoclonus Epilepsy, Action Myoclonus – Renal Failure (AMRF), CDKL5 deficiency disorder,
and Benign Familial Infantile Seizures (BFIS).

[0005] 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 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.

[0006] 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 interneurons of the hippocampus.

[0007] Despite the efforts made to date, there is a need in the art for improved compositions and methods for treating diseases mediated by premature termination codons, including Dravet syndrome.

SUMMARY OF THE INVENTION

[0008] The invention is based, in part, upon the discovery of tRNAs (*e.g.*, suppressor tRNAs), that permit 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. The invention is further based, in part, upon the discovery that a tRNA that permits an amino acid to be incorporated into a gene product encoded by a gene at a position that would otherwise result in a truncated gene product caused by a PTC in the gene, *e.g.*, a tRNA described herein, can be used to treat a disease mediated by a PTC in a gene in a subject.

[0009] Accordingly, in one aspect, the invention provides a tRNA comprising a nucleotide sequence set forth in **TABLE 2**. In certain embodiments, the tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 19-21, 37, 39, 40, 44, 179, 181, 182, and 186.

[0010] In certain embodiments, the 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-methoxy-carbonylmethyluridine, 5-methoxycarbonylmethyl-2-thiouridine, pseudouridine, dihydrouridine, 1-methyladenosine, and inosine.

[0011] In another aspect, the invention provides an expression vector comprising a nucleotide sequence encoding any of the foregoing tRNAs. In certain embodiments, the expression vector comprises 1, 2, 3, 4, or more than 4 copy numbers of the nucleotide sequence encoding the tRNA. In certain embodiments, the expression vector comprises a nucleotide sequence corresponding to a genomic DNA sequence flanking a wild-type tRNA gene. For example, in certain embodiments, the expression vector comprises a nucleotide sequence set forth in **TABLE 4**. In certain embodiments, the nucleotide sequence set forth in **TABLE 4** is selected from SEQ ID NOs: 869-888. In certain embodiments, the nucleotide sequence set forth in **TABLE 4** is operably linked to the nucleotide sequence encoding the tRNA. In certain embodiments, in the expression vector, the nucleotide sequence set forth in **TABLE 4** is 5' to the nucleotide sequence encoding the tRNA. In certain embodiments, in the expression vector, the nucleotide sequence set forth in **TABLE 4** is immediately 5' to (i.e., adjacent) the nucleotide sequence encoding the tRNA.

[0012] In another aspect, the invention provides an expression vector comprising a nucleotide sequence encoding a tRNA set forth in **TABLE 3**, wherein the expression vector comprises 1, 2, 3, 4, or more than 4 copy numbers of the nucleotide sequence encoding the tRNA. In certain embodiments, the tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 22, 35, 36, 38, 45, 178, 180, and 187.

[0013] In another aspect, the invention provides an expression vector comprising a nucleotide sequence encoding a tRNA set forth in **TABLE 3**, wherein the expression vector further comprises a nucleotide sequence set forth in **TABLE 4**. In certain embodiments, the tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 22, 35, 36, 38, 45, 178, 180, and 187. In certain embodiments, the nucleotide sequence set forth in **TABLE 4** is selected from SEQ ID NOs: 869-888. In certain embodiments, the nucleotide sequence set forth in **TABLE 4** is operably linked to the nucleotide sequence encoding the tRNA. In certain embodiments, in the expression vector, the nucleotide sequence set forth in **TABLE 4** is 5' to the nucleotide sequence encoding the tRNA. In certain embodiments, in the expression vector, the nucleotide sequence set forth in **TABLE 4** is immediately 5' to (i.e., adjacent) the nucleotide sequence encoding the tRNA.

[0014] In certain embodiments of any of the foregoing expression vectors, the expression vector is a viral vector, *e.g.*, a DNA virus vector, *e.g.*, an adeno-associated virus (AAV) vector.

[0015] In another aspect, the invention provides a pharmaceutical composition comprising any of the foregoing tRNAs or any of the foregoing expression vectors and a pharmaceutically acceptable excipient. In certain embodiments, the tRNA or expression vector is not conjugated to, or associated with, another moiety, *e.g.*, a carrier particle, *e.g.*, an aminolipid particle. In certain embodiments, the composition does not comprise a nanoparticle and/or an aminolipid delivery compound.

[0016] In another aspect, the invention 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 introducing into the cell an effective amount of any of the foregoing tRNAs or expression vectors, 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 certain embodiments of any of the foregoing methods, the gene is selected from a gene set forth in **TABLE 5** or **TABLE 6**. In certain embodiments, the gene is a SCN1A gene.

[0017] In another aspect, the invention 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 introducing into the cell an effective amount of a tRNA set forth in **TABLE 3** (*e.g.*, a tRNA comprising a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 22, 35, 36, 38, 45, 178, 180, and 187), or an expression vector comprising a nucleotide sequence encoding the tRNA, 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, wherein the gene is a gene set forth in **TABLE 5**. In certain embodiments, the gene is a SCN1A gene.

[0018] In certain embodiments of any of the foregoing methods, the cell contains less truncated gene product than a cell without the tRNA. In certain embodiments, the cell contains a greater amount of functional gene product than a cell without the tRNA.

[0019] In another aspect, the invention provides a method of increasing in a cell voltage-gated sodium channel activity encoded by a SCN1A gene containing a premature termination codon, the method comprising introducing into the cell an effective amount of any of the foregoing tRNAs or any of the foregoing expression vectors, thereby permitting an amino acid to be incorporated into the SCN1A gene product at a position that would otherwise result in a truncated SCN1A gene product caused by the premature termination codon.

[0020] In another aspect, the invention provides a method of increasing in a cell voltage-gated sodium channel activity encoded by a SCN1A gene containing a premature termination codon, the method comprising introducing into the cell an effective amount of a tRNA set forth in **TABLE 3** (*e.g.*, a tRNA comprising a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 22, 35, 36, 38, 45, 178, 180, and 187), or an expression vector comprising a nucleotide sequence encoding the tRNA, thereby permitting an amino acid to be incorporated into the SCN1A gene product at a position that would otherwise result in a truncated SCN1A gene product caused by the premature termination codon.

[0021] In certain embodiments of any of the foregoing methods, wherein the gene is a SCN1A gene, the SCN1A gene product produced with the tRNA is a functional SCN1A gene product. In certain embodiments, the functional SCN1A gene product has greater activity than the truncated SCN1A gene product. In certain embodiments, the functional SCN1A gene product is the Nav1.1 protein. In certain embodiments, the functional SCN1A gene product comprises the amino acid sequence of any one of SEQ ID NOs: 863-868.

[0022] In certain embodiments of any of the foregoing methods, the cell is a human cell. In certain embodiments, the cell is a central nervous system cell, *e.g.*, a neuron. In certain embodiments, the tRNA becomes aminoacylated in the cell.

[0023] In another aspect, the invention provides a method of treating a premature termination codon-mediated disorder in a subject in need thereof, wherein the subject has a gene with a premature termination codon, the method comprising administering to the subject an effective amount of any of the foregoing tRNAs or any of the foregoing expression vectors, thereby to treat the disorder in the subject. In certain embodiments, the disorder is selected from a disorder set forth in **TABLE 5** or **TABLE 6**.

[0024] In another aspect, the invention provides a method of treating a premature termination codon-mediated disorder in a subject in need thereof, wherein the subject has a gene with a premature termination codon, the method comprising administering to the subject an effective amount of a tRNA set forth in **TABLE 3** (*e.g.*, a tRNA comprising a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 22, 35, 36, 38, 45, 178, 180, and 187), or an expression vector comprising a nucleotide sequence encoding the tRNA, thereby to treat the disorder in the subject, wherein the disorder is a disorder set forth in **TABLE 5**.

[0025] In certain embodiments of any of the foregoing methods of treatment, the disorder is an epilepsy, *e.g.*, Dravet syndrome. In certain embodiments, the subject is human. In certain embodiments, the method further comprises administering an effective amount of another agent,

e.g., DIACOMIT[®] (stiripentol), EPIODOLEX[®] (cannabidiol), a ketogenic diet, ONFI[®] (clobazam), TOPAMAX[®] (topiramate), fenfluramine, or valproic acid, to the subject.

[0026] In certain embodiments of any of the foregoing methods, wherein the gene is a SCN1A gene, the premature termination codon in the SCN1A gene is caused by a mutation, or a
 5 combination of mutations, selected from c.664C>T, c.1129C>T, c.1492A>T, c.1624C>T, c.1738C>T, c.1837C>T, c.2134C>T, c.2593C>T, c.3637C>T, c.3733C>T, c.3985C>T, c.4573C>T, c.5656C>T, and c.5734C>T. In certain embodiments, the premature termination codon is caused by a mutation selected from c.1738C>T and c.3985C>T.

[0027] These and other aspects and features of the invention are described in the following
 10 detailed description and claims.

DESCRIPTION OF THE DRAWINGS

[0028] The invention can be more completely understood with reference to the following drawings.

[0029] **FIGURE 1** is a schematic representation of a transcript (*e.g.*, an SCN1A transcript)
 15 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-
 20 length protein.

[0030] **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
 25 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.

[0031] **FIGURE 3** is a schematic representation of a dual fluorescent reporter construct which contains three copies of a red fluorescent protein (tdTomato), a TEV protease, a 51 bp linker
 30 region comprising a PTC +/- 8 flanking codons, and three copies of a green fluorescent protein (EGFP). Expression is driven by a promoter for elongation factor EF-1 α located upstream of the

first copy of tdTomato. All copies of tdTomato, EGFP, and the TEV protease are separated from one another by TEV protease cleavage sites (Glu-Asn-Leu-Tyr-Phe-Gln-(Gly/Ser)).

[0032] FIGURE 4 is a graph depicting readthrough activity of Arg^{TCA} suppressor tRNAs in a Flp-In-293 cell line stably expressing a dual fluorescent reporter with the subject S-PTC linker region (SEQ ID NO: 29), which is derived from a clinically relevant SCN1A nonsense mutation linked to Dravet syndrome. Cells were transfected with the indicated Arg^{TCA} suppressor tRNAs (SEQ ID NOs: 1-25 and 35) and readthrough activity was measured by flow cytometry in two independent experiments at 24 hours post-transfection. "Parental" indicates the original Flp-In-293 cell line without a fluorescent reporter, "no PTC" indicates a Flp-In-293 cell line stably expressing a dual fluorescent reporter with a version of the subject S-PTC linker region that lacks a PTC (SEQ ID NO: 194), "EV" (empty vector) indicates cells transfected with an expression construct that does not contain a tRNA, "TCG" indicates cells transfected with an expression construct that contains a wild-type Arg-tRNA with a TCG anticodon. Readthrough activity is presented as the percentage of viable cells that that express both tdTomato and EGFP above background ("Double positive %"). Error bars represent the standard deviation of the data.

[0033] FIGURE 5 is a graph depicting readthrough activity of Arg^{TCA} suppressor tRNAs in a Flp-In-3T3 cell line stably expressing a dual fluorescent reporter with the R1407X-PTC linker region (SEQ ID NO: 30), which is derived from a clinically relevant SCN1A nonsense mutation linked to Dravet syndrome. Cells were transfected with the indicated Arg^{TCA} suppressor tRNAs (SEQ ID NOs: 1-25) and readthrough activity was measured by flow cytometry in three independent experiments at 24 hours post transfection. "Parental" indicates the original 3T3 cell line without a fluorescent reporter, "no PTC" indicates an 3T3 cell line stably expressing a dual fluorescent reporter with a version of the R1407X-PTC linker region that lacks a PTC (SEQ ID NO: 195), "Mock" indicates mock-transfected cells, "EV" (empty vector) indicates cells transfected with an expression construct that does not contain a tRNA or an EGFP reporter, "TCG" indicates cells transfected with an expression construct that contained a wild-type Arg-tRNA with a TCG anticodon. Readthrough activity is presented as the percentage of viable cells that that express both tdTomato and EGFP above background ("double positive %"). Error bars represent the standard deviation of the data.

[0034] FIGURE 6 is a graph depicting readthrough activity of Arg^{TCA} suppressor tRNAs in Flp-In-3T3 cells transiently transfected with a dual fluorescent reporter with the subject N-PTC linker region (SEQ ID NO: 28), which is derived from a clinically relevant SCN1A nonsense

- mutation linked to Dravet syndrome. Cells were co-transfected with the indicated Arg^{TCA} suppressor tRNAs (SEQ ID NOs: 1-25 and 35) and readthrough activity was measured by flow cytometry. "Mock" indicates mock-transfected cells, "no PTC" indicates cells transfected with a dual fluorescent reporter with a version of the subject N-PTC linker region that lacks a PTC (SEQ ID NO: 193), "EV" (empty vector) indicates cells co-transfected with an expression construct that does not contain a tRNA, "TCG" indicates cells co-transfected with an expression construct that contains a wild-type Arg-tRNA with a TCG anticodon. Readthrough activity was measured by flow cytometry and is presented as the percentage of viable cells that express both tdTomato and EGFP above background ("double positive %").
- 10 **[0035] FIGURE 7** is a graph depicting readthrough activity of the indicated Arg^{TCA} suppressor tRNAs (as measured by percent positive GFP cells) in Neuro-2a (N2a) and Flp-In-293 (293) cells. Cells were co-transfected with an expression construct containing an EGFP-R96X-TGA reporter (SEQ ID NO: 31) and an expression construct containing the indicated Arg^{TCA} suppressor tRNA (SEQ ID NOs: 1-22). "EGFP" indicates cells transfected with a version of the
- 15 EGFP reporter that lacks a PTC, "EGFP-PTC" indicates cells transfected with the EGFP-R96X-TGA reporter alone. EGFP expression was analyzed by flow cytometry at ~24 hours post transfection in 293 cells and ~48 hours post transfection in N2a cells. Readthrough activity is presented as the percentage of viable cells that express EGFP above background ("%GFP+") with all values normalized to cells expressing EGFP lacking a PTC.
- 20 **[0036] FIGURE 8A** is a depiction of an experimental approach to measure suppressor tRNA activity using a construct which contains an EGFP reporter with a PTC and a suppressor tRNA. Native termination codons are indicated as shaded circles, and premature termination codons are indicated as unshaded circles. A standard Arg-tRNA (with an anticodon that binds CGA) will result in no read-through of the PTC in EGFP, and a non-functional truncated EGFP protein. A
- 25 suppressor tRNA (with an anticodon that binds UGA) allows for read-through the PTC in EGFP resulting in full-length, functional EGFP protein. **FIGURE 8B** is a schematic representation of an exemplary reporter construct which contains EGFP with a PTC and 4 copies of a suppressor tRNA.
- 30 **[0037] FIGURE 9** depicts fluorescent images of Neuro-2a cells transfected with an expression construct containing an EGFP-R96X-TGA reporter (SEQ ID NO: 31) and the indicated Arg^{TCA} suppressor tRNA at the indicated copy number. Suppressor tRNAs are TCA-001 (SEQ ID NO: 11), TCA-113 (SEQ ID NO: 16), and TCA-115 (SEQ ID NO: 18). Each copy of the suppressor tRNA also contains 200 bps upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ

ID NO: 26) and 104 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 32). Images were taken ~24 hours post transfection. From left to right, the GFP controls are 1) wild-type EGFP alone, 2) wild-type EGFP on an expression construct including a single copy of the Arg^{TCA} suppressor tRNA #001 (SEQ ID NO: 11), 3) the EGFP-R96X-TGA reporter alone, and 4) the EGFP-R96X-TGA reporter on an expression construct including four copies of a wild-type Arg-tRNA with an unmodified TCG anticodon.

[0038] FIGURE 10 depicts fluorescent images of Flp-In-293 cells transfected with an expression construct containing an EGFP-R96X-TGA reporter (SEQ ID NO: 31) and the indicated Arg^{TCA} suppressor tRNA at the indicated copy number. Suppressor tRNAs are TCA-001 (SEQ ID NO: 11), TCA-113 (SEQ ID NO: 16), and TCA-115 (SEQ ID NO: 18). Each copy of the suppressor tRNA also contained 200 bps upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 26) and 104 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 32). Images were taken ~48 hours post transfection. From left to right, the GFP controls are 1) wild-type EGFP alone, 2) wild-type EGFP on an expression construct including a single copy of the Arg^{TCA} suppressor tRNA #001 (SEQ ID NO: 11), 3) the EGFP-R96X-TGA reporter alone, and 4) the EGFP-R96X-TGA reporter on an expression construct including four copies of a wild-type Arg-tRNA with an unmodified TCG anticodon.

[0039] FIGURE 11 shows fluorescence as measured by flow cytometry for Neuro-2a and Flp-In-293 cells transfected with an expression construct containing an EGFP-R96X-TGA reporter (SEQ ID NO: 177) and the Arg^{TCA} suppressor tRNA #001 (SEQ ID NO: 11). Expression constructs included one (1x), two (2x), or four (4x) copies of the suppressor tRNA in the context of either (i) a U6 promoter including 19 bps of upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 33) and 46 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 34) (“U6”), or (ii) 200 bps upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 26) and 200 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 27) (“Flank”). “Empty vector” indicates cells transfected with an expression construct that does not contain a tRNA or an EGFP reporter, “EF1a:EGFP” indicates cells transfected with a version of the EGFP reporter that lacks a PTC. Analysis was carried out ~48 hours post transfection. Data are presented as histograms displaying the frequency distribution of the data versus fluorescence intensity for viable cells expressing EGFP above background.

[0040] FIGURE 12 depicts percentage of EGFP positive cells and mean EGFP intensity in all viable cells as measured by flow cytometry for Neuro-2a cells transfected with an expression construct containing an EGFP-R96X-TGA reporter (SEQ ID NO: 177) and the Arg^{TCA} suppressor tRNA #001 (SEQ ID NO: 11) with the indicated copy number, in the context of either (i) a U6 promoter including 19 bps of upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 33) and 46 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 34) (“U6”), or (ii) 200 bps upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 26) and 200 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 27) (“Flank”). “Empty vector” indicates cells transfected with an expression construct that does not contain a tRNA or an EGFP reporter, “EF1a:EGFP” indicates cells transfected with a version of the EGFP reporter that lacks a PTC. Analysis was carried out ~48 hours post transfection. These plots summarize data from Neuro-2a cells in Figure 11.

[0041] FIGURE 13 shows fluorescence as measured by flow cytometry for Neuro-2a cells transfected with an expression construct containing an EGFP-R96X-TGA reporter (SEQ ID NO: 31) and the Arg^{TCA} suppressor tRNA #001 (SEQ ID NO: 11). Expression constructs included one (1x), two (2x), three (3x), or four (4x) copies of the suppressor tRNA. Each copy of the suppressor tRNA also contains 200 bps upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 26) and 104 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 32) (“Flank”). “CAG:EGFP” indicates cells transfected with a version of the EGFP reporter that lacks a PTC, “Mock” indicates cells transfected with the EGFP-R96X-TGA reporter alone. Analysis was carried out ~48 hours post transfection. Data are presented as histograms displaying the frequency distribution of the data versus fluorescence intensity for all viable cells (“G1 Gate”) and for viable cells expressing EGFP above background (“GFP+ Gate”).

[0042] FIGURE 14 shows fluorescence as measured by flow cytometry for Neuro-2a cells transfected with an expression construct containing an EGFP-R96X-TGA reporter (SEQ ID NO: 31) and the Arg^{TCA} suppressor tRNA #113 (SEQ ID NO: 16). Expression constructs included one (1x), two (2x), three (3x), or four (4x) copies of the suppressor tRNA. Each copy of the suppressor tRNA also contained 200 bps upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 26) and 104 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 32) (“Flank”). “CAG:EGFP” indicates cells transfected with a version of the EGFP reporter that lacks a PTC, “Mock” indicates cells transfected with the EGFP-PTC reporter alone. Analysis was carried out ~48 hours post transfection. Data are

presented as histograms displaying the frequency distribution of the data versus fluorescence intensity for all viable cells (“G1 Gate”) and for viable cells expressing EGFP above background (“GFP+ Gate”).

[0043] FIGURE 15 shows fluorescence as measured by flow cytometry for Neuro-2a cells transfected with an expression construct containing an EGFP-R96X-TGA reporter (SEQ ID NO: 31) and the Arg_{TCA} suppressor tRNA #115 (SEQ ID NO: 18). Expression constructs included one (1x), two (2x), three (3x), or four (4x) copies of the suppressor tRNA. Each copy of the suppressor tRNA also contained 200 bps upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 26) and 104 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 32) (“Flank”). “CAG:EGFP” indicates cells transfected with a version of the EGFP reporter that lacks a PTC, “Mock” indicates cells transfected with the EGFP-R96X-TGA reporter alone. Analysis was carried out ~48 hours post transfection. Data are presented as histograms displaying the frequency distribution of the data versus fluorescence intensity for all viable cells (“G1 Gate”) and for viable cells expressing EGFP above background (“GFP+ Gate”).

[0044] FIGURE 16 depicts percentage of EGFP positive cells in all viable cells (“% Cells GFP+”) and mean EGFP intensity in EGFP positive cells (“GFP+ Gate”) as measured by flow cytometry for Neuro-2a cells transfected with an expression construct containing an EGFP-R96X-TGA reporter (SEQ ID NO: 31) and the indicated suppressor tRNA at the indicated copy number. Suppressor tRNAs are TCA-001 (SEQ ID NO: 11), TCA-113 (SEQ ID NO: 16), and TCA-115 (SEQ ID NO: 18). “CAG:EGFP” indicates cells transfected with a version of the EGFP reporter that lacks a PTC, “CAG:EGFP-PTC” indicates cells transfected with the EGFP-R96X-TGA reporter alone. These plots summarize data from Figures 13-15.

[0045] FIGURE 17 depicts the percentage of EGFP positive cells as measured by flow cytometry for Neuro-2a cells transfected with an expression construct containing an EGFP-R96X-TGA reporter (SEQ ID NO: 31) and the indicated Arg_{TCA} suppressor tRNA with the indicated copy number and flanking sequence. Suppressor tRNAs are TCA-001 (SEQ ID NO: 11), TCA-113 (SEQ ID NO: 16), and TCA-115 (SEQ ID NO: 18). “U6” indicates a U6 promoter including 19 bps of upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 33) and 46 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 34), “Flank (±200 bps)” indicates 200 bps upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 26) and 200 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 27), “Flank (+200/-100 bps)” indicates 200 bps upstream flanking

genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 26) and 104 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 32) ("Flank"). "Mock" indicates mock transfected cells, "Empty Vector" indicates cells transfected with an expression construct that does not contain a tRNA or an EGFP reporter, "4x TCG" indicates cells transfected with an expression construct that contains an EGFP-R96X-TGA reporter and 4 copies of a wild-type Arg-tRNA with a TCG anticodon, "CAG:EGFP" indicates cells transfected with a version of the EGFP reporter that lacks a PTC. All data are normalized to positive control (CAG:EGFP). Plots show the percentage of viable cells that express GFP above background.

[0046] FIGURE 18 is a graph depicting readthrough activity of Gln^{TTA} suppressor tRNAs in two independently derived Flp-In-293 cell lines (#2 and #10) stably expressing a dual fluorescent reporter with the Dmd^{mdx}-PTC linker region (SEQ ID NO: 192), which is derived from a clinically relevant DMD nonsense mutation linked to Duchenne muscular dystrophy. Cells were transfected with the indicated Gln^{TTA} suppressor tRNAs (SEQ ID NOs: 36-48) and readthrough activity was measured by flow cytometry at 24 hours post transfection. "Parental" indicates the original Flp-In-293 cell line without a fluorescent reporter, "no PTC" indicates a Flp-In-293 cell line stably expressing a dual fluorescent reporter with a version of the Dmd^{mdx}-PTC linker region that lacks a PTC (SEQ ID NO: 191), "TTG" indicates cells transfected with an expression construct that contains a wild-type Gln-tRNA with a TTG anticodon. Readthrough activity was measured by flow cytometry and is presented as the percentage of viable cells that that express both tdTomato and EGFP above baseline ("%Double+ cells").

[0047] FIGURE 19 is a graph depicting readthrough activity of the indicated Gln^{TTA} suppressor tRNAs (SEQ ID NOs: 36-48) in Neuro-2a cells co-transfected with an expression construct containing an EGFP-Q69X-TAA reporter (SEQ ID NO: 175). "Mock" indicates mock-transfected cells, "EGFP" indicates cells transfected with a version of the EGFP reporter that lacks a PTC, "EV" (empty vector) indicates cells transfected with an expression construct that does not contain a tRNA or an EGFP reporter, "TTG" indicates cells co-transfected with the EGFP-Q69X-TAA reporter and an expression construct that contains a wild-type Gln-tRNA with a TTG anticodon. Readthrough activity was measured by flow cytometry and is presented as the percentage of viable cells that express GFP above background. Error bars represent the standard deviation of the data.

[0048] FIGURE 20 is a graph depicting readthrough activity of the indicated Gln^{CTA} suppressor tRNAs (SEQ ID NOs: 78-90) in Neuro-2a cells co-transfected with an expression construct containing an EGFP-Q69X-TAG reporter (SEQ ID NO: 176). "Mock" indicates mock-

transfected cells, "EGFP" indicated cells transfected with a version of the EGFP reporter that lacks a PTC, "EV" (empty vector) indicates cells transfected with an expression construct that does not contain a tRNA or an EGFP reporter, "TTG" indicates cells co-transfected with the EGFP-Q69X-TAG reporter and an expression construct that contains a wild-type Gln-tRNA
 5 with a TTG anticodon. Readthrough activity was measured by flow cytometry and is presented as the percentage of viable cells that express GFP above background. Error bars represent the standard deviation of the data.

[0049] FIGURE 21 depicts fluorescent images of Neuro-2a cells ~24 hours after transfection with an expression construct containing an EGFP-R96X-TGA reporter ("GFP-PTC") and either
 10 (i) including the Arg_{TCA} suppressor tRNA #115 (SEQ ID NO: 18) at the indicated copy number on the same construct, or (ii) treated with ataluren at the indicated concentration, (iii) treated with gentamicin at the indication concentration, or (iv) treated with G418 at the indicated concentration. For all experimental conditions, cell culture medium was replaced with fresh medium ~6 hours after transfection and the indicated drugs at the indicated concentrations were
 15 added at this point. Controls in the left column were transfected with an expression construct containing wild-type EGFP ("WT-GFP") and the indicated drug or Arg_{TCA} suppressor tRNA at the indicated copy number.

[0050] FIGURE 22 is a graph depicting percentage of GFP positive cells as measured by flow cytometry at ~48 hours post transfection. Neuro-2a cells were transfected with an expression
 20 construct containing an EGFP-R96X-TGA reporter ("GFP-PTC") and either (i) including the Arg_{TCA} suppressor tRNA #115 (SEQ ID NO: 18) at the indicated copy number on the same construct, or (ii) treated with ataluren at the indicated concentration, (iii) treated with gentamicin at the indication concentration, or (iv) treated with G418 at the indicated concentration. For all experimental conditions, cell culture medium was replaced with fresh medium ~6 hours after
 25 transfection and the indicated drugs at the indicated concentrations were added at this point. A reporter containing wildtype EGFP without a PTC ("WT-GFP") was used as a control. "Mock" indicates mock transfected cells. "4XTCG" indicates cells transfected with an expression construct that contains four copies of a wild-type Arg-tRNA with a TCG anticodon and the EGFP-R96X-TGA reporter. Plot shows the percentage of viable cells that express EGFP above
 30 background. The percentage of cells that expressed GFP ranged from 73.0 to 76.2% for the cells expressing the Arg_{TCA} suppressor, relative to 0.7 to 1.6% for negative controls, 0.9 to 4.9% for cells treated with ataluren, 1.2 to 6.5% for cells treated with gentamicin, and 6.7% to 26.7% for cells treated with G418.

[0051] FIGURE 23 is a graph depicting cell viability from Figure 22 as measured by flow cytometry at ~48 hours post transfection. Neuro-2a cells were transfected with an expression construct containing the EGFP-R96X-TGA reporter (“GFP-PTC”) and either (i) transfected with an expression construct including the Arg^{TCA} suppressor tRNA #115 (SEQ ID NO: 18) at the indicated copy number on the same construct, or (ii) treated with ataluren at the indicated concentration, (iii) treated with gentamicin at the indication concentration, or (iv) treated with G418 at the indicated concentration. For all experimental conditions, cell culture medium was replaced with fresh medium ~6 hour after transfection and the indicated drugs at the indicated concentrations were added at this point. A reporter containing wildtype GFP without a PTC (“WT-GFP”) was used as a control. “Mock” indicates mock transfected cells. “4XTCG” indicates cells transfected with an expression construct that contains four copies of a wild-type Arg-tRNA with a TCG anticodon and the EGFP-R96X-TGA reporter. Cell viability was assessed by flow cytometry using 7-Amino Actinomycin D (7-AAD), a membrane impermeant dye that is generally excluded from viable cells.

[0052] FIGURE 24 is a graph depicting readthrough activity of the indicated Gln^{CTA} suppressor tRNAs (SEQ ID NOs: 178-190) in Flp-In-293 cells transiently co-transfected with the indicated dual fluorescent reporter constructs that contain linker regions (SEQ ID NOs: 889, 891, and 893) derived from three clinically relevant Gln(Q)-to-TAG PTC mutations in SCN1A (W1397*, S1505*, and Q1810*) that are linked to Dravet syndrome. “Mock” indicates mock-transfected cells, “No PTC” indicates cells transfected with versions of the dual fluorescent reporter constructs lacking a PTC (SEQ ID Nos: 890, 892, and 894), “EV” (empty vector) indicates cells transfected with an expression construct that does not contain a tRNA or a fluorescent reporter, “TTG” indicates cells co-transfected with the indicated dual fluorescent reporter construct and an expression construct that contains a wild-type Gln-tRNA with a TTG anticodon. Readthrough activity was measured by flow cytometry at ~24 hours post-transfection and is presented as the percentage of viable cells that that express both tdTomato and EGFP above baseline (“% Double+ cells”).

[0053] FIGURE 25 is a graph depicting readthrough activity of the indicated Gln^{CTA} suppressor tRNAs (SEQ ID NOs: 178-190) in a Flp-In-293 cell line containing an integrated dual fluorescent reporter construct that contains a linker region (SEQ ID NO: 889) which is derived from a clinically relevant PTC mutation in SCN1A (W1397*) that is linked to Dravet syndrome. “Mock” indicates mock-transfected cells, “RFP-EGFP” indicates a Flp-In-293 cell line containing an integrated version of the dual fluorescent reporter construct lacking a PTC (SEQ ID NO: 890), “EV” (empty vector) indicates cells transfected with an expression construct that

does not contain a tRNA or an EGFP reporter, "TTG" indicates cells transfected with a wild-type Gln-tRNA with a TTG anticodon. Readthrough activity was measured by flow cytometry at ~24 hours post transfection and is presented as the percentage of cells that that express both tdTomato and EGFP above background (" % Double+ cells").

5 [0054] **FIGURE 26A** shows fluorescence as measured by flow cytometry for Neuro-2a cells transfected with an expression construct containing an EGFP-R96X-TGA reporter (SEQ ID NO: 31) and a single copy of the Arg^{TCA} suppressor tRNA #115 (SEQ ID NO: 18), in the context of either (i) 200 bps upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 26) and 104 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 32) 10 ("Flank300"), (ii) 20 bps upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 895) and 17 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 896) ("Flank20"), (iii) 10 bps upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 897) and 17 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 896) ("Flank10"), or (iv) 0 bps upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 and 17 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 896) ("Flank0"). Details of the expression vectors are shown in **TABLE 11**. "GFP-PTC" indicates cells transfected with the EGFP-R96X-TGA reporter alone (SEQ ID NO: 31), "GFP" indicates cells transfected with a version of the EGFP reporter that lacks a PTC. Readthrough activity was measured by flow cytometry at ~24 hours post transfection. Data are presented as 20 histograms displaying the frequency distribution of the data versus fluorescence intensity for cells expressing EGFP above background. **FIGURE 26B** shows the percentage of EGFP positive cells in all viable cells (" % GFP+") and mean EGFP intensity in viable cells expressing EGFP above background ("Mean GFP Signal") for the cells depicted in **FIGURE 26A**.

25 [0055] **FIGURE 27** is a schematic representation of the constructs used to test the impact of a 5' leader sequence on the read-through of a premature termination codon (PTC) by a suppressor tRNA. Constructs contain (i) a 100 bp 5' leader sequence derived from genomic DNA located upstream of tRNA genes that are highly expressed in HEK293 cells, (ii) a single copy of either the Arg^{TCA} suppressor tRNA #115 (SEQ ID NO: 18) or the Gln^{TTA} suppressor tRNA #163 (SEQ ID NO: 45), and (iii) an RNA polymerase III termination signal ("Term").

30 [0056] **FIGURE 28** is a graph depicting the readthrough activity of the indicated Arg^{TCA} suppressor tRNA expression constructs as measured by flow cytometry in Flp-In-293 cells at ~24 hours post transfection. Cells were co-transfected with constructs containing (i) an EGFP-R96X-TGA reporter (SEQ ID NO: 31) and (ii) the Arg^{TCA} suppressor tRNA #115 (SEQ ID NO:

18) in the context of the indicated 100 bps upstream genomic DNA leader sequences (SEQ ID NOs: 869-888). "WT-EGFP" indicates cells transfected with a reporter containing wild-type GFP without a PTC, "EV" (empty vector) indicates cells co-transfected with an expression construct that does not contain a tRNA or an EGFP reporter, and "26/27" indicates cells co-transfected with the ArgTCA suppressor tRNA #115 (SEQ ID NO: 18) in the context of 200 bps upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 26) and 200 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 27). The plot shows the percentage of viable cells that express EGFP above background. The percentage of cells that expressed EGFP ranged from 17.2 to 33.7% for the cells expressing the ArgTCA suppressor, relative to 0.4% for the empty vector control.

[0057] FIGURE 29 is a graph depicting the readthrough activity of the indicated ArgTCA suppressor tRNA expression constructs as measured by flow cytometry in Flp-In-293 cells at ~24 hours post transfection. Cells were co-transfected with constructs containing (i) an EGFP-R96X-TGA reporter (SEQ ID NO: 31) and (ii) the ArgTCA suppressor tRNA #115 (SEQ ID NO: 18) in the context of the indicated 100 bps upstream genomic DNA leader sequences (SEQ ID NOs: 869-888). "WT-EGFP" indicates cells transfected with a reporter containing wild-type GFP without a PTC, "EV" (empty vector) indicates cells co-transfected with an expression construct that does not contain a tRNA or an EGFP reporter, and "26/27" indicates cells co-transfected with the ArgTCA suppressor tRNA #115 (SEQ ID NO: 18) in the context of 200 bps upstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 26) and 200 bps of downstream flanking genomic DNA from tRNA-Arg-TCG-1-1 (SEQ ID NO: 27). The plot shows mean EGFP intensity in cells expressing EGFP above background. The mean EGFP intensity ranged from 1990 to 4319 for the cells expressing the ArgTCA suppressor, relative to 602 for the empty vector control.

[0058] FIGURE 30 is a graph depicting the readthrough activity of the indicated GlnTTA suppressor tRNA expression constructs as measured by flow cytometry in Flp-In-293 cells at ~24 hours post transfection. Cells were co-transfected with constructs containing (i) an EGFP-Q69X-TAA reporter (SEQ ID NO: 175) and (ii) the GlnTTA suppressor tRNA #163 (SEQ ID NO: 45) in the context of the indicated 100 bps upstream genomic DNA leader sequences (SEQ ID NOs: 869-888). "WT-EGFP" indicates cells transfected with a reporter containing wild-type GFP without a PTC, "EV" (empty vector) indicates cells co-transfected with an expression construct that does not contain a tRNA or an EGFP reporter, and "173/174" indicates cells co-transfected with the GlnTTA suppressor tRNA #163 (SEQ ID NO: 45) in the context of 200 bps upstream flanking genomic DNA from tRNA-Gln-TTG-1-1 (SEQ ID NO: 173) and 200 bps of

downstream flanking genomic DNA from tRNA-Gln-TTG-1-1 (SEQ ID NO: 174). The plot shows the percentage of viable cells that express EGFP above background. The percentage of cells that expressed EGFP ranged from 21.4 to 35.7% for the cells expressing the Gln^{TTA} suppressor, relative to 0.3% for the empty vector control.

5 [0059] FIGURE 31 is a graph depicting the readthrough activity of the indicated Gln^{TTA} suppressor tRNA expression constructs as measured by flow cytometry in Flp-In-293 cells at ~24 hours post transfection. Cells were co-transfected with constructs containing (i) an EGFP-Q69X-TAA reporter (SEQ ID NO: 175) and (ii) the Gln^{TTA} suppressor tRNA #163 (SEQ ID NO: 45) in the context of the indicated 100 bps upstream genomic DNA leader sequences (SEQ ID NOs: 869-888). “WT-EGFP” indicates cells transfected with a reporter containing wild-type GFP without a PTC, “EV” (empty vector) indicates cells co-transfected with an expression construct that does not contain a tRNA or an EGFP reporter, and “173/174” indicates cells co-transfected with the Gln^{TTA} suppressor tRNA #163 (SEQ ID NO: 45) in the context of 200 bps upstream flanking genomic DNA from tRNA-Gln-TTG-1-1 (SEQ ID NO: 173) and 200 bps of downstream flanking genomic DNA from tRNA-Gln-TTG-1-1 (SEQ ID NO: 174). The plot shows mean EGFP intensity in cells expressing EGFP above background. The mean EGFP intensity ranged from 1702 to 3822 for the cells expressing the Gln^{TTA} suppressor, relative to 387 for the empty vector control.

[0060] FIGURE 32 is table summarizing the results of FIGURES 28-31, where the values have been normalized to cells transfected with wild-type GFP without a PTC.

[0061] FIGURE 33 is a graph depicting the percentage of EGFP positive cells as measured by flow cytometry in three independent experiments at ~48 hours post transfection. Neuro-2a cells were transfected with an expression construct containing the EGFP-Q69X-TAA reporter (“EGFP-PTC”) (SEQ ID NO: 175) and either (i) including the Gln^{TTA} suppressor tRNA #002 (SEQ ID NO: 36) at the indicated copy number on the same construct, or (ii) treated with ataluren at the indicated concentration, (iii) treated with gentamicin at the indicated concentration, or (iv) treated with G418 at the indicated concentration. For all experimental conditions, cell culture medium was replaced with fresh medium ~6 hours after transfection and the indicated drugs at the indicated concentrations were added at this point. A reporter containing wildtype EGFP without a PTC (“EGFP”) was used as a control. “Mock” indicates mock transfected cells, “no tRNA” indicates cells transfected with the indicated EGFP expression construct alone, “4X-Gln-TTG” indicates cells transfected with an expression construct that contains four copies of a wild-type Gln-tRNA with a TTG anticodon and the

EGFP-Q69X-TAA reporter. The plot shows the percentage of viable cells that express GFP above background. Error bars represent the standard deviation of the data. The percentage of cells that expressed GFP ranged from 64.7 to 67.3% for the cells expressing the Gln^{TTA} suppressor, relative to 0.2 to 0.7% for negative controls, 0.4 to 0.6% for cells treated with
 5 ataluren, 0.4% for cells treated with gentamicin, and 1.7% to 4.5% for cells treated with G418.

[0062] FIGURE 34 is a graph depicting cell viability from FIGURE 33 as measured by flow cytometry at ~48 hours post transfection. Neuro-2a cells were transfected with an expression construct containing the EGFP-Q69X-TAA reporter (“EGFP-PTC”) (SEQ ID NO: 175) and either (i) including the Gln^{TTA} suppressor tRNA #002 (SEQ ID NO: 36) at the indicated copy
 10 number on the same construct, or (ii) treated with ataluren at the indicated concentration, (iii) treated with gentamicin at the indication concentration, or (iv) treated with G418 at the indicated concentration. For all experimental conditions, cell culture medium was replaced with fresh medium ~6 hours after transfection and the indicated drugs at the indicated concentrations were added at this point. A reporter containing wildtype EGFP without a PTC (“EGFP”) was used as
 15 a control. “Mock” indicates mock transfected cells, “no tRNA” indicates cells transfected with the indicated EGFP expression construct alone, “4X-Gln-TTG” indicates cells transfected with an expression construct that contains four copies of a wild-type Gln-tRNA with a TTG anticodon and the EGFP-Q69X-TAA reporter. Cell viability was assessed by flow cytometry using 7-Amino Actinomycin D (7-AAD), a membrane impermeant dye that is generally excluded from
 20 viable cells.

[0063] FIGURE 35 is a graph depicting the percentage of EGFP positive cells as measured by flow cytometry in three independent experiments at ~48 hours post transfection. Neuro-2a cells were transfected with an expression construct containing the EGFP-Q69X-TAG reporter (“EGFP-PTC”) (SEQ ID NO: 176) and either (i) including the Gln^{CTA} suppressor tRNA #196
 25 (SEQ ID NO: 178) at the indicated copy number on the same construct, or (ii) treated with ataluren at the indicated concentration, (iii) treated with gentamicin at the indication concentration, or (iv) treated with G418 at the indicated concentration. For all experimental conditions, cell culture medium was replaced with fresh medium ~6 hour after transfection and the indicated drugs at the indicated concentrations were added at this point. A reporter
 30 containing wildtype EGFP without a PTC (“EGFP”) was used as a control. “Mock” indicates mock transfected cells, “no tRNA” indicates cells transfected with the indicated EGFP expression construct alone, “4X-Gln-TTG” indicates cells transfected with an expression construct that contains four copies of a wild-type Gln-tRNA with a TTG anticodon and the EGFP-Q69X-TAA reporter. Plot shows the percentage of viable cells that express GFP above

background. The percentage of cells that expressed GFP ranged from 73.1 to 78.2% for the cells expressing the Gln^{CTA} suppressor, relative to 0.5 to 0.8% for negative controls, 0.5 to 0.7% for cells treated with ataluren, 0.5 to 1.1% for cells treated with gentamicin, and 13.6% to 20.6% for cells treated with G418.

- 5 [0064] FIGURE 36 is a graph depicting cell viability from FIGURE 35 as measured by flow cytometry at ~48 hours post transfection. Neuro-2a cells were transfected with an expression construct containing the EGFP-Q69X-TAG reporter (“EGFP-PTC”) (SEQ ID NO: 176) and either (i) including the Gln^{CTA} suppressor tRNA #196 (SEQ ID NO: 178) at the indicated copy number on the same construct, or (ii) treated with ataluren at the indicated concentration, (iii) 10 treated with gentamicin at the indication concentration, or (iv) treated with G418 at the indicated concentration. For all experimental conditions, cell culture medium was replaced with fresh medium ~6 hour after transfection and the indicated drugs at the indicated concentrations were added at this point. A reporter containing wildtype EGFP without a PTC (“EGFP”) was used as a control. “Mock” indicates mock transfected cells, “no tRNA” indicates cells transfected with 15 the indicated EGFP expression construct alone, “4X-Gln-TTG” indicates cells transfected with an expression construct that contains four copies of a wild-type Gln-tRNA with a TTG anticodon and the EGFP-Q69X-TAA reporter. Cell viability was assessed by flow cytometry using 7-Amino Actinomycin D (7-AAD), a membrane impermeant dye that is generally excluded from viable cells.
- 20 [0065] FIGURE 37A is a Western blot depicting rescue of full-length SCN1A protein expression by a suppressor tRNA. Flp-In-293 cells were transfected with an expression construct containing mouse SCN1A with an Arg(R)-to-TGA PTC (R1407X) and a 3xFLAG tag peptide (DYKDHD-G-DYKDHD-I-DYKDDDDK) at the C-terminus (SEQ ID NO: 899) (“SCN1a (R1407*)”) and either (i) co-transfected with an expression construct containing the 25 Arg^{TCA} suppressor tRNA #115 (SEQ ID NO: 18) (“Arg>TGA #115”), or (ii) treated with ataluren at the indicated concentration, (iii) treated with gentamicin at the indication concentration, or (iv) treated with G418 at the indicated concentration. “SCN1a (w.t.)” indicates cells transfected with an expression construct containing wild-type mouse SCN1A and a 3xFLAG tag peptide at the C-terminus (SEQ ID NO: 898). Protein was isolated at 24 hours 30 post transfection and SCN1A was detected using a monoclonal anti-FLAG M2 antibody. Molecular weights based on protein molecular weight markers are indicated to the left of the gel. FIGURE 37B is a quantification of the Western blot shown in FIGURE 37A. The intensity of bands corresponding to the size of full-length SCN1A protein was measured using ImageJ and all intensity values were normalized to wild-type SCN1A protein (“SCN1a (w.t.)” lane). Cells

co-transfected with expression vectors containing mutant SCN1A and the Arg^{TCA} suppressor tRNA expressed greater than 70% of the full-length SCN1A expressed by cells transfected with an expression construct containing wild-type SCN1A.

[0066] **FIGURE 38A** is a Western blot depicting rescue of full-length SCN1A protein expression by suppressor tRNAs. Flp-In-293 cells were co-transfected with (i) an expression construct containing mouse SCN1A with an Arginine-to-TGA PTC (R1407X) and a 3xFLAG tag peptide (DYKDHD-G-DYKDHD-I-DYKDDDDK) at the C-terminus (SEQ ID NO: 899) (“SCN1a (R1407*)”) and (ii) either the #104 Arg^{TCA} suppressor tRNA (SEQ ID NO: 6) (“Sup #104”), the #106 Arg^{TCA} suppressor tRNA (SEQ ID NO: 8) (“Sup #106”), or the Arg^{TCA} suppressor tRNA #115 (SEQ ID NO: 18) (“Sup #115”). “SCN1a (w.t.)” indicates cells transfected with an expression construct containing wild-type mouse SCN1A and a 3xFLAG tag peptide at the C-terminus (SEQ ID NO: 898). Protein was isolated at 24 hours post transfection and SCN1A was detected using a monoclonal anti-FLAG M2 antibody. Molecular weights based on protein molecular weight markers are indicated to the left of the gel. **FIGURE 38B** is a quantification of the Western blot shown in **FIGURE 38A**. The intensity of bands corresponding to the size of full-length SCN1A protein was measured using ImageJ and all intensity values were normalized to wild-type SCN1A protein (“SCN1a (w.t.)” lane). Cells co-transfected with expression vectors containing mutant SCN1A and the Arg^{TCA} suppressor tRNA expressed greater than 30% (Sup #104), greater than 60% (Sup #106), or greater than 70% (Sup #115) of the full-length SCN1A expressed by cells transfected with an expression construct containing wild-type SCN1A.

[0067] **FIGURE 39A** is a Western blot depicting rescue of full-length SCN1A protein expression by a suppressor tRNA. Flp-In-293 cells were cultured in 6-well cell culture plates and co-transfected with (i) an expression construct containing mouse SCN1A with an Arginine-to-TGA PTC (R1407X) and a 3xFLAG tag peptide (DYKDHD-G-DYKDHD-I-DYKDDDDK) at the C-terminus (SEQ ID NO: 899) (“SCN1a (R1407*)”) and (ii) the Arg^{TCA} suppressor tRNA #115 (SEQ ID NO: 18) (“Arg>TGA #115”) at the indicated concentrations. 1x indicates 400 ng of the Arg^{TCA} suppressor tRNA construct per well, 0.3x indicates 133 ng of the Arg^{TCA} suppressor tRNA construct per well, 0.1x indicates 40 ng of the Arg^{TCA} suppressor tRNA construct per well, and 0.03x indicates 13 ng of the Arg^{TCA} suppressor tRNA construct per well. “SCN1a (w.t.)” indicates cells transfected with an expression construct containing wild-type mouse SCN1A and a 3xFLAG tag peptide at the C-terminus (SEQ ID NO: 898). Protein was isolated at 24 hours post transfection and SCN1A was detected using a monoclonal anti-FLAG M2 antibody. Molecular weights based on protein molecular weight markers are indicated to the

left of the gel. **FIGURE 39B** is a quantification of the Western blot shown in **FIGURE 39A**. The intensity of bands corresponding to the size of full-length SCN1A protein was measured using ImageJ and all intensity values were normalized to wild-type SCN1A protein (“SCN1a (w.t.)” lane).

5 **[0068] FIGURE 40** is a schematic representation of constructs that were packaged into AAV-PHP.eB capsids. Construct 262 contains wild-type EGFP driven by an EF1a promoter. Construct 269 contains EGFP-R96X-TGA driven by an EF1a promoter (SEQ ID NO: 177) and two copies of the Arg^{TCA} suppressor tRNA #115 (SEQ ID NO: 18) (“TCA-115”) in the context of 55 bps upstream flanking genomic DNA from tRNA-Tyr-GTA-5-1 (SEQ ID NO: 900). Both
10 constructs contain 5' and 3' ITR sequences from AAV2, which provide cis-acting elements for AAV replication and packaging.

[0069] FIGURE 41 depicts readthrough activity of suppressor tRNAs delivered by AAV. AAV-PHP.eB containing the constructs shown in **FIGURE 40** was produced by Vigene Biosciences. 48 hours prior to AAV transduction, 293 cells were pre-transfected with an expression construct
15 containing the LY6A gene, which is required for robust transduction by AAV-PHP.eB. Cells were transduced at an MOI of 1E5 vg/cell. Where indicated, cells were also transfected with an expression construct containing the EGFP-R96X-TGA reporter (SEQ ID NO: 31) and an expression construct containing the Arg^{TCA} suppressor tRNA #115 (SEQ ID NO: 18). At 72 hours post transduction, the EGFP signal was quantified by immunofluorescence. Live cell
20 images were captured on an EVOS FL Auto 2 imaging system. CellProfiler software was used to segment and extract the integrated EGFP intensity for nuclei in each image. These values were averaged across all nuclei in each condition. Background average integrated EGFP intensity from the negative control condition was subtracted from each of these averages, and all values were normalized. The plot depicts the normalized % GFP intensity with all values
25 normalized to cells transduced with AAV-PHP.eB containing construct 262.

[0070] FIGURE 42 shows fluorescence as measured by flow cytometry for Neuro-2a cells used in ribosome footprint profiling analysis. Cells were transfected with either (i) an expression construct containing an EGFP-R96X-TGA reporter (SEQ ID NO: 177) and the Arg^{TCA} suppressor tRNA #001 (SEQ ID NO: 11) on the same construct (“Arg^{TCA} EGFP-PTC”) or (ii) an
30 expression construct containing a version of the EGFP reporter that lacks a PTC (“WT-EGFP”).

[0071] FIGURE 43 depicts fold change in 3' UTR read density distributions (as determined by ribosome profiling) between the two Neuro-2a cells populations shown in **FIGURE 42**. Cells were transfected with either (i) an expression construct containing an EGFP-R96X-TGA reporter

(SEQ ID NO: 177) and the Arg^{TCA} suppressor tRNA #001 (SEQ ID NO: 11) on the same construct (“Arg^{TCA} EGFP-PTC”) or (ii) an expression construct containing a version of the EGFP reporter that lacks a PTC (“WT-EGFP”). Cells were lysed at ~48 hours post transfection and subjected to ribosome footprint profiling. Raw reads were trimmed of adapters using

5 Trimmomatic then depleted for non-coding RNA by aligning against Ensembl's mouse mm10 ncRNA reference using bowtie2. Remaining reads were aligned against UCSC's mm10 mouse reference assembly, again using bowtie2. Multi-mapping reads were discarded. The resulting final set of aligned reads was quantified using the RiboProfiling package in R and custom

10 Python scripting. Python was used to generate plots examining 3' UTR occupancy and fold change for each gene with 20 or more uniquely mapping reads, and the distributions for genes with each native stop codon were compared using the 2 sample Kolmogorov-Smirnov test.

DETAILED DESCRIPTION

[0072] The invention is based, in part, upon the discovery of tRNAs (*e.g.*, suppressor tRNAs), that permit an amino acid to be incorporated into a gene product encoded by a gene in a

15 mammalian cell at a position that would otherwise result in a truncated gene product caused by a premature termination codon (PTC) in the gene. The invention is further based, in part, upon the discovery that a tRNA that permits an amino acid to be incorporated into a gene product encoded by a gene at a position that would otherwise result in a truncated gene product caused by a PTC in the gene can be used to treat a disease mediated by a PTC in a gene in a subject.

20 [0073] Accordingly, in one aspect, the invention provides a tRNA (*e.g.*, an isolated tRNA) comprising a nucleotide sequence set forth in **TABLE 2**.

[0074] In another aspect, the invention provides an expression vector comprising a nucleotide sequence encoding a tRNA, *e.g.*, as shown in **TABLES 1-3**. In certain embodiments, the expression vector comprises 1, 2, 3, 4, or more than 4 copy numbers of the nucleotide sequence

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

[0075] In another aspect, the invention provides a pharmaceutical composition comprising any

30 of the foregoing tRNAs or any of the foregoing expression vectors and a pharmaceutically acceptable excipient.

[0076] In another aspect, the invention 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 introducing into the cell an effective amount of a tRNA (*e.g.*, as shown in TABLES 1-3 below, *e.g.*, comprising SEQ ID NO: 6-9, 11, 16-22, 35-40, 44, 45, 178-182, 186, or 187), or an expression vector comprising a nucleotide sequence encoding the tRNA, 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.

[0077] In certain embodiments of any of the foregoing methods, the cell contains less truncated gene product than a cell without the tRNA. For example, in certain embodiments, the cell contains less than about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% of the truncated gene product relative to a cell without the tRNA. In certain embodiments, the cell contains from about 5% to about 80%, about 5% to about 60%, about 5% to about 40%, about 5% to about 20%, about 5% to about 10%, about 10% to about 80%, about 10% to about 60%, about 10% to about 40%, about 10% to about 20%, about 20% to about 80%, about 20% to about 60%, about 20% to about 40%, about 40% to about 80%, about 40% to about 60%, or about 60% to about 80% of the truncated gene product relative to a cell without the tRNA. In certain embodiments, there is no detectable truncated gene product in the cell. Truncated gene product amount or expression may be measured by any method known in the art, for example, Western blot or ELISA.

[0078] In certain embodiments, the cell contains a greater amount of functional gene product than a cell without the tRNA. For example, in certain embodiments, the method increases the amount of functional gene product in a cell, tissue, or subject by about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 110%, about 120%, about 130%, about 140%, about 150%, about 160%, about 170%, about 180%, about 190%, about 200%, about 250%, about 300%, about 350%, about 400%, about 450%, or about 500% relative to a cell, tissue, or subject without the tRNA. In certain embodiments, the method increases the amount of functional gene product in a cell, tissue, or subject, by from about 20% to about 200%, about 20% to about 180%, about 20% to about 160%, about 20% to about 140%, about 20% to about 120%, about 20% to about 100%, about 20% to about 80%, about 20% to about 60%, about 20% to about 40%, about 40% to about 200%, about 40% to about 180%, about 40% to about 160%, about 40% to about 140%, about 40% to about 120%, about 40% to about 100%, about 40% to about 80%, about 40% to about 60%, about 60% to about 200%, about 60% to about 180%, about 60% to about 160%, about 60% to about 140%, about 60% to about 120%, about 60% to about 100%, about 60% to about 80%, about 80% to about 200%, about 80% to about 180%, about 80% to about 160%, about 80% to about 140%, about 80% to about 120%, about 80% to about 100%, about 100% to about

200%, about 100% to about 180%, about 100% to about 160%, about 100% to about 140%, about 100% to about 120%, about 120% to about 200%, about 120% to about 180%, about 120% to about 160%, about 120% to about 140%, about 140% to about 200%, about 140% to about 180%, about 140% to about 160%, about 160% to about 200%, about 160% to about 180%, or about 180% to about 200% relative to a cell, tissue, or subject without the tRNA. Functional gene product amount or expression may be measured by any method known in the art, for example, Western blot or ELISA.

[0079] In certain embodiments, the tRNA permits an amino acid to be incorporated into the gene product at a position corresponding to a premature termination codon (*i.e.* the tRNA permits read-through of the premature termination codon), but the tRNA does not permit a substantial amount of amino acid to be incorporated into a gene product at a position corresponding to a native stop codon (*i.e.*, the tRNA does not permit read-through of a native stop codon). For example, in certain embodiments, a disclosed tRNA does not increase read-through of a native stop codon (or all native stop codons) in a cell, tissue, or subject, or increases read-through by less than about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 20%, about 30%, about 40%, or about 50%, relative to a cell, tissue, or subject that has not been contacted with the tRNA. Read-through of a native stop codon may be measured by any method known in the art, for example, ribosome profiling as described in Example 13 herein.

[0080] In certain embodiments of any of the foregoing methods, the gene is selected from a gene set forth in **TABLE 5** or **TABLE 6**. In certain embodiments, the gene is a SCN1A gene.

[0081] In another aspect, the invention provides a method of expressing in a cell a functional SCN1A gene product encoded by a SCN1A gene containing a premature termination codon, the method comprising introducing into the cell an effective amount of a tRNA (*e.g.*, as shown in **TABLES 1-3** below, *e.g.*, comprising SEQ ID NO: 6-9, 11, 16-22, 35-40, 44, 45, 178-182, 186, or 187), or an expression vector comprising a nucleotide sequence encoding the tRNA, thereby permitting an amino acid to be incorporated into the SCN1A gene product at a position that would otherwise result in a truncated SCN1A gene product caused by the premature termination codon.

[0082] In another aspect, the invention provides a method of increasing in a cell voltage-gated sodium channel activity encoded by a SCN1A gene containing a premature termination codon, the method comprising introducing into the cell an effective amount of a tRNA (*e.g.*, as shown in **TABLES 1-3** below, *e.g.*, comprising SEQ ID NO: 6-9, 11, 16-22, 35-40, 44, 45, 178-182, 186, or 187), or an expression vector comprising a nucleotide sequence encoding the tRNA, thereby

permitting an amino acid to be incorporated into the SCN1A gene product at a position that would otherwise result in a truncated SCN1A gene product caused by the premature termination codon.

[0083] In another aspect, the invention provides a method of treating a premature termination
5 codon-mediated disorder in a subject in need thereof wherein the subject has a gene with a premature termination codon, the method comprising administering to the subject an effective amount of a tRNA (*e.g.*, as shown in TABLES 1-3 below, *e.g.*, comprising SEQ ID NO: 6-9, 11, 16-22, 35-40, 44, 45, 178-182, 186, or 187), or an expression vector comprising a nucleotide sequence encoding the tRNA, thereby to treat the disorder in the subject. In certain
10 embodiments, the disorder is selected from a disorder set forth in TABLE 5 or TABLE 6.

[0084] In another aspect, the invention provides a method of treating Dravet syndrome in a subject in need thereof wherein the subject has a SCN1A gene with a premature termination codon, the method comprising administering to the subject an effective amount of a tRNA (*e.g.*,
as shown in TABLES 1-3 below, *e.g.*, comprising SEQ ID NO: 6-9, 11, 16-22, 35-40, 44, 45,
15 178-182, 186, or 187), or an expression vector comprising a nucleotide sequence encoding the tRNA, thereby to treat Dravet syndrome in the subject.

I. tRNAs and Suppressor tRNAs

[0085] During protein synthesis, a transfer RNA (tRNA) delivers an amino acid to a ribosome for incorporation into a growing protein (polypeptide) chain. tRNAs typically are about 70 to
20 100 nucleotides in length, and active tRNAs contain a 3' CCA sequence that may be transcribed into the tRNA during its synthesis or may be added later during post-transcriptional processing. During aminoacylation, the amino acid that is attached to a given tRNA molecule is covalently attached to the 2' or 3' hydroxyl group of the 3'-terminal ribose to form an aminoacyl-tRNA (aa-tRNA). It is understood that an amino acid can spontaneously migrate from the 2'-hydroxyl
25 group to the 3'-hydroxyl group and vice versa, but it is incorporated into a growing protein chain at the ribosome from the 3'-OH position. A loop at the other end of the folded aa-tRNA molecule contains a sequence of three bases known as the anticodon. When this anticodon sequence hybridizes or base-pairs with a complementary three-base codon sequence in a ribosome-bound messenger RNA (mRNA), the aa-tRNA binds to the ribosome and its amino
30 acid is incorporated into the polypeptide chain being synthesized by the ribosome. Because all tRNAs that base-pair with a specific codon are aminoacylated with a single specific amino acid, the translation of the genetic code is effected by tRNAs. Each of the 61 non-termination codons

in an mRNA directs the binding of its cognate aa-tRNA and the addition of a single specific amino acid to the growing polypeptide chain being synthesized by the ribosome.

[0086] tRNAs are generally highly conserved and are often functional across species.

Accordingly, a tRNA derived from a bacterial tRNA, a non-mammalian eukaryotic tRNA, or a
5 mammalian (*e.g.*, human) tRNA may be useful in the practice of the invention. Nucleotide sequences encoding naturally occurring human tRNAs are known and generally available to those of skill in the art through sources such as Genbank. See also Sprinzl *et al.* (2005) NUCLEIC ACIDS RES. 33: D139-40; Buckland *et al.* (1996) GENOMICS 35(1):164-71; Schimmel *et al.* (Eds.) (1979) "Transfer-RNA: Structure, Properties, and Recognition," Cold Spring Harbor
10 Laboratory; Agris (1983) "The Modified Nucleosides of Transfer RNA, II," Alan R. Liss Inc. tRNAs are generally highly conserved and are often functional across species.

[0087] Suppressor tRNAs are modified tRNAs that insert a suitable amino acid at a mutant site, *e.g.*, a PTC, in protein encoding gene. The use of the word in suppressor is based on the fact, that under certain circumstance, the modified tRNA "suppresses" the phenotypic effect of the
15 coding mutation. Suppressor tRNAs typically contain a mutation (modification) in either the anticodon, changing codon specificity, or at some position that alters the aminoacylation identity of the tRNA.

[0088] In certain embodiments, a tRNA (*e.g.*, a suppressor tRNA) contains a modified anticodon region, such that the modified anticodon hybridizes with a different codon than the
20 corresponding naturally occurring anticodon. In certain embodiments, the modified anticodon hybridizes with a termination codon, *e.g.*, a PTC, and as a result, the tRNA incorporates an amino acid into a gene product rather than terminating protein synthesis. In certain embodiments, the modified anticodon hybridizes with a premature termination codon and, and as a result, the tRNA incorporates an amino acid into a gene product at a position that would
25 otherwise result in a truncated gene product caused by the premature termination codon.

[0089] In certain embodiments, a tRNA comprises an anticodon that hybridizes to a codon selected from UAG (*i.e.*, an "amber" termination codon), UGA (*i.e.*, an "opal" termination codon), and UAA (*i.e.*, an "ochre" termination codon). In certain embodiments, the anticodon hybridizes to a codon selected from UGA to UAA. In certain embodiments, the anticodon
30 hybridizes to UGA. In certain embodiments, a tRNA comprises an anticodon that hybridizes to a non-standard termination codon, *e.g.*, a 4-nucleotide codon (See, for example, Moore *et al.* (2000) J. MOL. BIOL. 298:195, and Hohsaka *et al.* (1999) J. AM. CHEM. SOC. 121:12194).

[0090] In certain embodiments, the tRNA is aminoacylated or is capable of being aminoacylated with any natural amino acid. For example, a tRNA may be capable of being aminoacylated with alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. In certain embodiments the tRNA is capable of being aminoacylated with serine, leucine, glutamine, or arginine. In certain embodiments the tRNA is capable of being aminoacylated with glutamine or arginine. In certain embodiments the tRNA is capable of being aminoacylated with arginine.

[0091] In certain embodiments, the tRNA (i) comprises an anticodon that hybridizes to a codon as indicated in **TABLE 1**, and (ii) is aminoacylated or is capable of being aminoacylated with an amino acid as indicated in **TABLE 1**.

TABLE 1

codon: UAG amino acid: alanine	codon: UGA amino acid: alanine	codon: UAA amino acid: alanine
codon: UAG amino acid: arginine	codon: UGA amino acid: arginine	codon: UAA amino acid: arginine
codon: UAG amino acid: asparagine	codon: UGA amino acid: asparagine	codon: UAA amino acid: asparagine
codon: UAG amino acid: aspartic acid	codon: UGA amino acid: aspartic acid	codon: UAA amino acid: aspartic acid
codon: UAG amino acid: cysteine	codon: UGA amino acid: cysteine	codon: UAA amino acid: cysteine
codon: UAG amino acid: glutamine	codon: UGA amino acid: glutamine	codon: UAA amino acid: glutamine
codon: UAG amino acid: glutamic acid	codon: UGA amino acid: glutamic acid	codon: UAA amino acid: glutamic acid
codon: UAG amino acid: glycine	codon: UGA amino acid: glycine	codon: UAA amino acid: glycine
codon: UAG amino acid: histidine	codon: UGA amino acid: histidine	codon: UAA amino acid: histidine
codon: UAG amino acid: isoleucine	codon: UGA amino acid: isoleucine	codon: UAA amino acid: isoleucine
codon: UAG	codon: UGA	codon: UAA

amino acid: leucine	amino acid: leucine	amino acid: leucine
codon: UAG amino acid: lysine	codon: UGA amino acid: lysine	codon: UAA amino acid: lysine
codon: UAG amino acid: methionine	codon: UGA amino acid: methionine	codon: UAA amino acid: methionine
codon: UAG amino acid: phenylalanine	codon: UGA amino acid: phenylalanine	codon: UAA amino acid: phenylalanine
codon: UAG amino acid: proline	codon: UGA amino acid: proline	codon: UAA amino acid: proline
codon: UAG amino acid: serine	codon: UGA amino acid: serine	codon: UAA amino acid: serine
codon: UAG amino acid: threonine	codon: UGA amino acid: threonine	codon: UAA amino acid: threonine
codon: UAG amino acid: tryptophan	codon: UGA amino acid: tryptophan	codon: UAA amino acid: tryptophan
codon: UAG amino acid: tyrosine	codon: UGA amino acid: tyrosine	codon: UAA amino acid: tyrosine
codon: UAG amino acid: valine	codon: UGA amino acid: valine	codon: UAA amino acid: valine

[0092] In certain embodiments, the tRNA comprises, consists essentially of, or consists of a nucleotide sequence shown in **TABLE 2**. In certain embodiments, the tRNA comprises, consists essentially of, or consists of a nucleotide sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a nucleotide sequence shown in **TABLE 2**. In certain embodiments, the tRNA comprises, consists essentially of, or consists of a nucleotide sequence selected from SEQ ID NOs: 19-21, 37, 39, 40, 44, 179, 181, 182, and 186, or a nucleotide sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a nucleotide sequence selected from SEQ ID NOs: 19-21, 37, 39, 40, 44, 179, 181, 182, and 186.

5 It is understood that, throughout the description, in each instance where a tRNA comprises, consists essentially of, or consists of a nucleotide sequence including one or more thymines (T), a tRNA is also contemplated that comprises, consists essentially of, or consists of the same nucleotide sequence including a uracil (U) in place of one or more of the thymines (T), or a uracil (U) in place of all the thymines (T). Similarly, in each instance where a tRNA comprises,

10

consists essentially of, or consists of a nucleotide sequence including one or more uracils (U), a tRNA is also contemplated that comprises, consists essentially of, or consists of a nucleotide sequence including a thymine (T) in place of the one or more of the uracils (U), or a thymine (T) in place of all the uracils (U).

5

TABLE 2

SEQ ID NO	Suppressor tRNA Sequence (anticodon lowercase)
2	GGGCCAGTGGCGCAATGGATAACGCGTCTGACTtcaGATCAGAAGATTGTAG GTTTCGACTCCTACCTGGCTCG
5	GGCCGCGTGGCCTAATGGATAAGGCGTCTGATTtcaGATCAGAAGATTGGGG GTTTCGAGTCCCTTCGTGGTCG
14	GACCACGTGGCCTAACGGATAAGGCGTCTGACTtcaGATCAGAAGATTGAGG GTTTCGAATCCCTTCGTGGTTA
15	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTtcaAGTGACGAGAAAGCGA TTCAAAGGTTGTGGGTTCGAATCCCACCAGAGTCG
19	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTtcaAGCATGATTGAGAGAT TCAAAGGTTGCGGGTTCGAGTCCCGCCAGAGTCG
20	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTtcaAATTCAAAGGTTGCGG GTTTCGAGTCCCGCCAGAGTCG
21	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTtcaAGACAAATGGAGGCAT TCAAAGGTTGTGGGTTCGAGTCCCACCAGAGTCG
23	GTCTCTGTGGCGCAATGGACGAGCGCGCTGGACTtcaAATCCAGAGGTTCTG GGTTCGAGTCCCGGCAGAGATG
24	GGCTCTGTGGAGCAATGGATAGCACATTGGACTtcaAGCATGACCGAGAGAT TCAAAGGTTGCGGGTTCGAGTCCCACCAGAGTTG
25	GGCTCTGTGGAGCAATGGATAGCACATTGGACTtcaAATTCAAAGGTTGCGG GTTTCGAGTCCCACCAGAGTTG
37	GGTCCCATGGTGTAAATGGTTAGCACTCTGGACTttaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
39	GGTTCATGGTGTAAATGGTTAGCACTCTGGACTttaAATCCAGCGATCCGAG TTCAAATCTCGGTGGAACCT
40	GGTTCATGGTGTAAATGGTTAGCACTCTGGACTttaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT

41	GGTTCCATGGTGTAAATGGTGAGCACTCTGGACTttaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
42	GGTTCCATGGTGTAAATGGCTAGCACTCTGGACTttaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGATTT
43	GGTTCCATGGTGTAAATGGTTAGCACTCTGGACTttaAATCCAGCCATACAAG TTCAAATCTCAGTGGAACCT
44	GGTTCCTTGGTGTAAAGATGAGCACTCTGGATTttaAATCCAGCGATCAGAGT TCAAATCTCGGTGGGACCT
46	GGCCCCATGGTGTAAATGGTTAGCACTCTGGACTttaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
47	GGTCTCATGGTGTAAATGGTTAGCACACTGGACTttaAGTCCAGCAATCCGAG TTCGAGTCTTGGTGAGACCA
48	GGACCCATGGTGTAAATGGTTAGCACTCTGGACTttaAATCCAGCAATCCAAG TTCAAATCTCGGTGGGACCT
49	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTctaGCTATGGCTTCCTCG CTCTGAGGGTTCTGGTCTCCCCTGGAGGCGTGGGTTCGAATCCCACCTTCTGA CA
50	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTctaGCTTAGCTTCCCTGT CTGGGGATTCTGGTCTCCGTATGGAGGCGTGGGTTCGAATCCCACCTTCTGAC A
51	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTctaGGTGACAAGCCTTAC CTACGGGTGTTCTGGTCTCCGAATGGAGGCGTGGGTTCGAATCCCACCTTCTG ACA
52	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTctaGCGTTCGCTTCCTCT ACTGAGGGTTCTGGTCTCCGTGTGGAGGCGTGGGTTCGAATCCCACCTTCTGA CA
53	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTtcaGCTATGGCTTCCTCG CTCTGAGGGTTCTGGTCTCCCCTGGAGGCGTGGGTTCGAATCCCACCTTCTGA CA
54	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTtcaGCTTAGCTTCCCTGT CTGGGGATTCTGGTCTCCGTATGGAGGCGTGGGTTCGAATCCCACCTTCTGAC A

55	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTtcaGGTGACAAGCCTTAC CTACGGGTGTTCTGGTCTCCGAATGGAGGCGTGGGTTCGAATCCCACCTTCTG ACA
56	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTtcaGCGTTCGCTTCCTCT ACTGAGGGTTCTGGTCTCCGTGTGGAGGCGTGGGTTCGAATCCCACCTTCTGA CA
57	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTttaGCTATGGCTTCCTCG CTCTGAGGGTTCTGGTCTCCCCTGGAGGCGTGGGTTCGAATCCCACCTTCTGA CA
58	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTttaGCTTAGCTTCCTGT CTGGGGATTCTGGTCTCCGTATGGAGGCGTGGGTTCGAATCCCACCTTCTGAC A
59	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTttaGGTGACAAGCCTTAC CTACGGGTGTTCTGGTCTCCGAATGGAGGCGTGGGTTCGAATCCCACCTTCTG ACA
60	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTttaGCGTTCGCTTCCTCT ACTGAGGGTTCTGGTCTCCGTGTGGAGGCGTGGGTTCGAATCCCACCTTCTGA CA
61	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTctaGAGTTACTAGAATAGT GATCCTTAGGTCGCTGGTTCGAATCCGGCTCGAAGGA
62	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTctaGTCAGTACAATATGGT AATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
63	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTctaGGCTTGTGGCTGTGGA CATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
64	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTctaGCTAACTCCCCGTTAG AAGACATCCTTAGGTCGCTGGTTCGACTCCGGCTCGAAGGA
65	CTTTCGATAGTTTCAGTTGGTAGAGCGGAGGACTctaGAGTATTAACGTTGGT GATCCTTAGGTCGCTGGTTCGAGTCCGGCTCGAAGGA
66	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTttaGAGTTACTAGAATAGT GATCCTTAGGTCGCTGGTTCGAATCCGGCTCGAAGGA
67	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTttaGTCAGTACAATATGGT AATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
68	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTttaGGCTTGTGGCTGTGGA CATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA

69	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTttaGCTAACTCCCCGTTAG AAGACATCCTTAGGTCGCTGGTTCGACTCCGGCTCGAAGGA
70	CTTTCGATAGTTTCAGTTGGTAGAGCGGAGGACTttaGAGTATTAACGTTGGT GATCCTTAGGTCGCTGGTTCGAGTCCGGCTCGAAGGA
71	GGGGGTATAGCTCAGTGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
72	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGATGCCCCCT
73	GGGGGTATAGCTCAGGGGTAGAGTATTTGGCTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
74	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCTTGG TTCAAATCCAGGTGTCCCCCT
75	GGGGGTATAGCTCAGAGGTAGAGCATTTGACTtcaGATCAAGAGATCTCTGG TTCAAATCCAGGTGCCCCCT
76	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTAG TTCAAATCCAGGTGCCCCCT
77	GGTGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGATCCCTGG TTCGAATCCAGGTGCCCCCT
78	GGGGGTATAACTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
79	TGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
80	GGGGGTATAGCTCAGAGGAAGAGCATTTGACTtcaGATCAAGAGGTCCCTGA TTCAAATCCAGGTGCCCCCT
81	GGGGGTAAAGCTCAGGGGTAGAGCATTTGACTtcaGATTAAGAGGTCCCTGG TTCAAATCCAGGTACCCCCCT
83	GGGGTTATAGCTCAGGTGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
84	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCACGAGGTCCCTGG TTCAAATCGAGGTGCCCCCT
85	GGGGGTATAGCTCAGGGGTGGAGCATTTGACTtcaGATCAAGGGGTCCCTGT TTCAAATCCAGGTGCCCCCT
86	GGGGGTATAGCTCAGTGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCCGG TTCAAATCCGGGTGCCCCCT

88	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCGGGTGCCCCCT
89	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTACCCCCCT
91	GGGGGCATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCCGG TTCAAATCCGGGTGCTCCCT
92	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATTAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
94	TCCCTGGTGGTCTAGTGGTTAGGATTTGGCGCTctaACCGCCGCGGCCTGGG TTCGATTCCCGGTCAGGGAA
95	TCCCTGGTGGTCTAGTGGTTAGGCTTTGGTGCTctaACCTCCATGGCCCAGG TTTGATTCCTGGTCAGGGAA
97	TCCCTGGTGGTCTAGTGGTTAGGATTTGGCGCTttaACCGCCGCGGCCTGGG TTCGATTCCCGGTCAGGGAA
98	TCCCTGGTGGTCTAGTGGTTAGGCTTTGGTGCTttaACCTCCATGGCCCAGG TTTGATTCCTGGTCAGGGAA
100	TCCCATATGGTCTAGCGGTTAGGATTCCTGGTTctaACCCAGGCGGCCCGGG TTCGACTCCCGGTATGGGAA
103	TCCCATATGGTCTAGCGGTTAGGATTCCTGGTTttaACCCAGGCGGCCCGGG TTCGACTCCCGGTATGGGAA
105	GTTTCCGTAGTGTAGTGGTTAGCGCGTTCGCCTtcaAAAGCGAAAGGTCCCC GGTTCGAAACCGGGCGGAAACA
107	GCATTGGTAGTTCAATGGTAGAATTCTCGCCTtcaACGCGGGTGACCCGGGT TCGATTCCCGGCCAATGCA
108	GCATTGGTGGTTCAATGGTAGAATTCTCGCCTtcaACGCGGGTGACCCGGGT TCGATTCCCGGCCAATGCA
109	GCATTGGTGGTTCAATGGTAGAATTCTCGCCTtcaACTCGGGTGACCCGGGT TCGATTCCCGGCCAATGCA
112	GCATTGGTGGTTCAAGTGGTAGAATTCTCGCCTtcaACGCGGGAGGCCCGGGT TTGATTCCCGGCCAATGCA
113	GCATTGGTGGTTCAAGTGGTAGAATTCTCGCCTtcaACGCGGGAGGCCCGGGT TCGGTTCCCGGCCAATGCA
115	GGTAGCGTGGCCGAGCGGTCTAAGGCGCTGGATTctaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCTGCCA

116	GGTAGTGTGGCCGAGCGGTCTAAGGCGCTGGATTctaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCTGCCA
118	GGTAGCGTGGCCGAGCGGTCTAAGGCGCTGGATTtcaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCTGCCA
119	GGTAGTGTGGCCGAGCGGTCTAAGGCGCTGGATTtcaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCTGCCA
121	GGTAGCGTGGCCGAGCGGTCTAAGGCGCTGGATTttaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCTGCCA
122	GGTAGTGTGGCCGAGCGGTCTAAGGCGCTGGATTttaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCTGCCA
124	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTctaGTTCTGGTCTCCCCT GGAGGCGTGGGTTCGAATCCCACCTTCTGACA
126	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTctaGTTCTGGTCTCCGAA TGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
127	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTctaGTTCTGGTCTCCGTG TGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
128	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTtcaGTTCTGGTCTCCCCT GGAGGCGTGGGTTCGAATCCCACCTTCTGACA
130	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTtcaGTTCTGGTCTCCGAA TGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
131	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTtcaGTTCTGGTCTCCGTG TGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
132	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTttaGTTCTGGTCTCCCCT GGAGGCGTGGGTTCGAATCCCACCTTCTGACA
134	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTttaGTTCTGGTCTCCGAA TGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
135	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTttaGTTCTGGTCTCCGTG TGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
136	GTCAGGATGGCCGAGCGGTCTAAGGCGCTGCGTTctaGTGCGAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCACCTCCTGACA
137	GTCAGGATGGCCGAGCGGTCTAAGGCGCTGCGTTctaGTGCGAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCACCTTCTGACA
138	GTCAGGATGGCCGAGTGGTCTAAGGAGCTGTGTTctaGTGCGAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCACCTCCTGACA

139	GTCAGGATGGCCGAGCAGTCTAAGGCACTGCGTTctaGTCGCAGTCTCCCCCT GGAGGCGTGGATTTCGAATCCCACCTCCTGACA
140	GTCAGGATGGCCGAGCGGTCTAAGGCGCTGCGTTtcaGTCGCAGTCTCCCCCT GGAGGCGTGGGTTCGAATCCCACCTCCTGACA
141	GTCAGGATGGCCGAGCGGTCTAAGGCGCTGCGTTtcaGTCGCAGTCTCCCCCT GGAGGCGTGGGTTCGAATCCCACCTTCTGACA
142	GTCAGGATGGCCGAGTGGTCTAAGGAGCTGTGTTtcaGTCGCAGTCTCCCCCT GGAGGCGTGGGTTCGAATCCCACCTCCTGACA
143	GTCAGGATGGCCGAGCAGTCTAAGGCACTGCGTTtcaGTCGCAGTCTCCCCCT GGAGGCGTGGATTTCGAATCCCACCTCCTGACA
144	GTCAGGATGGCCGAGCGGTCTAAGGCGCTGCGTTttaGTCGCAGTCTCCCCCT GGAGGCGTGGGTTCGAATCCCACCTCCTGACA
145	GTCAGGATGGCCGAGCGGTCTAAGGCGCTGCGTTttaGTCGCAGTCTCCCCCT GGAGGCGTGGGTTCGAATCCCACCTTCTGACA
146	GTCAGGATGGCCGAGTGGTCTAAGGAGCTGTGTTttaGTCGCAGTCTCCCCCT GGAGGCGTGGGTTCGAATCCCACCTCCTGACA
147	GTCAGGATGGCCGAGCAGTCTAAGGCACTGCGTTttaGTCGCAGTCTCCCCCT GGAGGCGTGGATTTCGAATCCCACCTCCTGACA
148	ACCAGAATGGCCGAGTGGTTAAGGCGTTGGACTctaGATCCAATGGATTTAT ATCCGCGTGGGTTCGAACCCCCACTTCTGGTA
149	ACCAGGATGGCCGAGTGGTTAAGGCGTTGGACTctaGATCCAATGGACATAT GTCTGCGTGGGTTCGAACCCCCACTCCTGGTA
150	ACTGGGATGGCTGAGTGGTTAAGGCGTTGGACTctaGATCCAATGGGCGGTT GCCTGCGTGGGTTCGAACCCCCACTCCCAGTA
151	GATGGGATGGCTGAGAGGTTAAGGCTTTGGACTctaGATCCAATGGGCAGAT GCCTGCGTGGGTTTGAACCCCCACTCCCAATA
152	ACCAGAATGGCCGAGTGGTTAAGGCGTTGGACTtcaGATCCAATGGATTTAT ATCCGCGTGGGTTCGAACCCCCACTTCTGGTA
153	ACCAGGATGGCCGAGTGGTTAAGGCGTTGGACTtcaGATCCAATGGACATAT GTCTGCGTGGGTTCGAACCCCCACTCCTGGTA
154	ACTGGGATGGCTGAGTGGTTAAGGCGTTGGACTtcaGATCCAATGGGCGGTT GCCTGCGTGGGTTCGAACCCCCACTCCCAGTA
155	GATGGGATGGCTGAGAGGTTAAGGCTTTGGACTtcaGATCCAATGGGCAGAT GCCTGCGTGGGTTTGAACCCCCACTCCCAATA

156	ACCAGAATGGCCGAGTGGTTAAGGCGTTGGACTttaGATCCAATGGATTTAT ATCCGCGTGGGTTCGAACCCCACCTTCTGGTA
157	ACCAGGATGGCCGAGTGGTTAAGGCGTTGGACTttaGATCCAATGGACATAT GTCTGCGTGGGTTCGAACCCCACCTCCTGGTA
158	ACTGGGATGGCTGAGTGGTTAAGGCGTTGGACTttaGATCCAATGGGCGGTT GCCTGCGTGGGTTCGAACCCCACCTCCCAGTA
159	GATGGGATGGCTGAGAGGTTAAGGCTTTGGACTttaGATCCAATGGGCAGAT GCCTGCGTGGGTTTGAACCCCACCTCCCAATA
161	GGTAGTGTGGCCGAGCGGTCTAAGGCGCTGGATTctaGCTCCAGTCTCTTCG GAGGCGTGGGTTCGAATCCCACCACTGCCA
164	GGTAGTGTGGCCGAGCGGTCTAAGGCGCTGGATTtcaGCTCCAGTCTCTTCG GAGGCGTGGGTTCGAATCCCACCACTGCCA
167	GGTAGTGTGGCCGAGCGGTCTAAGGCGCTGGATTttaGCTCCAGTCTCTTCG GAGGCGTGGGTTCGAATCCCACCACTGCCA
169	GCCCAGCTAGCTCAGTTGGTAGAGCGTGGGACTctaAATCCTAGGGTCGTGG GTTCGAACCCCACGTTGGGCG
170	GCCCAGCTAGCTCAGTCTGTAGAGCATGAGACTctaAGTCTCAGGGTCATGG GTTGGAGCCCCATGTTGTGCA
171	GCCTAGCTAGTTCAGTCGGTAGAGCATGAGACTctaAATCTCAGGTTTCATGA GTTTGAGCCCCATGTTGGTTTGGCA
172	CCCCGGCTAGCTCAGTCAGTAGAGCTTGAGAATctaAATCTCAGGGTCGTGG GTTGGAGCCCCACGTTGGGCG
179	GGTTCCATGGTGTAAATGGTTAGCACTCTGGACTctaAATCCAGCGACCCGAG TTCAAATCTCGGTGGGACCT
181	GGTTCCATGGTGTAAATGGTTAGCACTCTGGACTctaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
182	GGTTCCATGGTGTAAATGGTGAGCACTCTGGACTctaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
183	GGTTCCATGGTGTAAATGGCTAGCACTCTGGACTctaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGATTT
184	GGTTCCATGGTGTAAATGGTTAGCACTCTGGACTctaAATCCAGCCATAACAAG TTCAAATCTCAGTGGAACCT
185	GGTTCCTTGGTGTAAAGATGAGCACTCTGGATTctaAATCCAGCGATCAGAGT TCAAATCTCGGTGGGACCT

186	GGTCCCATGGTGTAAATGGTTAGCACTCTGGACTctaAATCCAGCAATCTGAG TTCAAATCTCGGTGGGACCT
188	GGTCTCATGGTGTAAATGGTTAGCACACTGGACTctaAGTCCAGCAATCCGAG TTCGAGTCTTGGTGAGACCA
189	GGACCCATGGTGTAAATGGTTAGCACTCTGGACTctaAATCCAGCAATCCAAG TTCAAATCTCGGTGGGACCT
190	GTTTCCATGGTGTAAATGGTTGGCACTCTGGACTctaAATCCAGCAATCCAAG TTCAAGTCTCTGTGGGACCT
196	GCCCGGCTAGCTCAGTCGGTAGAGCATGGGACTctaAATCCCAGGGTCGTGG GTTCGAGCCCCACGTTGGGCG
197	GCCCGGCTAGCTCAGTCGGTAGAGCATGAGACTctaAATCTCAGGGTCGTGG GTTCGAGCCCCACGTTGGGCG
198	GCCCAGCTAGCTCAGTCTGTAGAGCATGAGACTctaAATCTCAGGGTCGTGA GTTCGAGCCCCACGTTGGGTG
199	GCCCAGATAGCTCAGTGGGTAGAGCATGAGACTctaAATCTCAGGGTCATGG GTTCATGCCCCATGTTGGGTA
200	GTCTTGCTGGCTCAGTCGGTACAGCATGGGACTctaAATCCCAGGGTCGTGG GTTCGAGCTCCACGTTGGGTA
201	GCCTGGCTAGCTCAGTCCATAGAGCATGGGACTctaAATCCCAGGGTCATGG GTTCGAGCCCCATATTAGGCA
202	GCCCAGCTAGCTTAGTTGGTAGAGCATGAGACTctaAATCTCAGAGTCATGG GTTCAGGCCTCATGTTTGGCA
203	AACCTGGCTAGGTCAGTTGGTAGATCATGAGACTctaAATCTCAGGGTCATG GGTTCAAGCCCCATGTTGGTTT
204	GCCCAGCTAGCTCAGTTGGTAGAGCGTGGGACTttaAATCCTAGGGTCGTGG GTTTGAACCCCCACGTTGGGCG
205	GCCCAGCTAGCTCAGTCTGTAGAGCATGAGACTttaAGTCTCAGGGTCATGG GTTGGAGCCCCATGTTGTGCA
206	GCCTAGCTAGTTCAGTCGGTAGAGCATGAGACTttaAATCTCAGGTTTCATGA GTTTGAGCCCCATGTTGGTTTGGCA
207	CCCGGCTAGCTCAGTCAGTAGAGCTTGAGAAtttaAATCTCAGGGTCGTGG GTTGGAGCCCCACGTTGGGCG
208	GCCCGGCTAGCTCAGTCGGTAGAGCATGGGACTttaAATCCCAGGGTCGTGG GTTCGAGCCCCACGTTGGGCG

209	GCCCGGCTAGCTCAGTCGGTAGAGCATGAGACTttaAATCTCAGGGTCGTGG GTTCGAGCCCCACGTTGGGCG
210	GCCCAGCTAGCTCAGTCTGTAGAGCATGAGACTttaAATCTCAGGGTCGTGA GTTCGAGCCCCACGTTGGGTG
211	GCCCAGATAGCTCAGTGGGTAGAGCATGAGACTttaAATCTCAGGGTCATGG GTTTCATGCCCCATGTTGGGTA
212	GTCTGCTGGCTCAGTCGGTACAGCATGGGACTttaAATCCCAGGGTCGTGG GTTCGAGCTCCACGTTGGGTA
213	GCCTGGCTAGCTCAGTCCATAGAGCATGGGACTttaAATCCCAGGGTCATGG GTTCGAGCCCCATATTAGGCA
214	GCCCAGCTAGCTTAGTTGGTAGAGCATGAGACTttaAATCTCAGAGTCATGG GTTCAAGCCTCATGTTTGGCA
215	AACCTGGCTAGGTCAAGTTGGTAGATCATGAGACTttaAATCTCAGGGTCATG GGTTCAAGCCCCATGTTGGTTT
216	GCCCGGATAGCTCAGTCGGTAGAGCATCAGACTctaAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTCTGGGCG
218	GCCTGGATAGCTCAATTGGTAGAGCATCAGACTctaAATCTGAGGGTTCAGG GTTCAAGTCCCTGTTCTAGGCG
219	GCCCAGCCAGCTCAGTAGGTAGAGTATGAGACTctaAATCTCAGGGTGGTGG GTTCGAGCCCCATGTTGGGGG
220	TGTGGTGTAGCTCAGTCGGTAGAGCATCAGACTctaAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTCTGGGTGCCAAA
221	GCCCGGATAGCTCAGTCGGTAGAGCATCAGACTttaAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTCTGGGCG
223	GCCTGGATAGCTCAATTGGTAGAGCATCAGACTttaAATCTGAGGGTTCAGG GTTCAAGTCCCTGTTCTAGGCG
224	GCCCAGCCAGCTCAGTAGGTAGAGTATGAGACTttaAATCTCAGGGTGGTGG GTTCGAGCCCCATGTTGGGGG
225	TGTGGTGTAGCTCAGTCGGTAGAGCATCAGACTttaAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTCTGGGTGCCAAA
228	GTAGTCGTGGCCAAGTGAGTAAGGCAATGGACTctaAATCCATTGGGGTCTC CCAGCACAGGTTCAAATCCTGCTGACTATG
231	GTAGTCGTGGCCAAGTGAGTAAGGCAATGGACTtcaAATCCATTGGGGTCTC CCAGCACAGGTTCAAATCCTGCTGACTATG

234	GTAGTCGTGGCCAAGTGAGTAAGGCAATGGACTttaAATCCATTGGGGTCTC CCAGCACAGGTTCAAATCCTGCTGACTATG
237	GCTGTGATGGCCGAGTGGTTAAGGCGTTGGACTctaAATCCAATGGGTTCTT CCCGCGCAGGTTCAAATCCTGCTCACAGCG
240	GCTGTGATGGCCGAGTGGTTAAGGCGTTGGACTtcaAATCCAATGGGTTCTT CCCGCGCAGGTTCAAATCCTGCTCACAGCG
243	GCTGTGATGGCCGAGTGGTTAAGGCGTTGGACTttaAATCCAATGGGTTCTT CCCGCGCAGGTTCAAATCCTGCTCACAGCG
245	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTctaAATCCATTGTGCTCTG CACGCATGGGTTTCAATCCCATCCTCGTCG
246	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTctaAATCCATTGTGCTTTG CACGCGTGGGTTTCAATCCCATCCTCGTCG
247	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTctaAATCCATTGTGCTCTG CACGCGTGGGTTTCAATCCCATCCTCGTCG
248	GATGAGGTGGCCGAGTGGTTAAGGCGATGGACTctaAATCCATTGTGCTCTG CACGCATGGGTTTCAATCCCATCCTCATCG
250	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTtcaAATCCATTGTGCTCTG CACGCATGGGTTTCAATCCCATCCTCGTCG
251	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTtcaAATCCATTGTGCTTTG CACGCGTGGGTTTCAATCCCATCCTCGTCG
252	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTtcaAATCCATTGTGCTCTG CACGCGTGGGTTTCAATCCCATCCTCGTCG
253	GATGAGGTGGCCGAGTGGTTAAGGCGATGGACTtcaAATCCATTGTGCTCTG CACGCATGGGTTTCAATCCCATCCTCATCG
255	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTttaAATCCATTGTGCTCTG CACGCATGGGTTTCAATCCCATCCTCGTCG
256	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTttaAATCCATTGTGCTTTG CACGCGTGGGTTTCAATCCCATCCTCGTCG
257	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTttaAATCCATTGTGCTCTG CACGCGTGGGTTTCAATCCCATCCTCGTCG
258	GATGAGGTGGCCGAGTGGTTAAGGCGATGGACTttaAATCCATTGTGCTCTG CACGCATGGGTTTCAATCCCATCCTCATCG
259	GCTGAAATAGCTCAGTTGGGAGAGCATTAGACTctaGATCTAAAGGTCCCTG GTTTGATCCCGGGTTTCGGCA

260	GCTGAAATAGCTCAGTTGGGAGAGCATTAGACTtcaGATCTAAAGGTCCCTG GTTTGATCCCGGGTTTCGGCA
261	GCTGAAATAGCTCAGTTGGGAGAGCATTAGACTttaGATCTAAAGGTCCCTG GTTTGATCCCGGGTTTCGGCA
265	GACCTCGTGGCGCAATGGTAGCGGTCTGACTctaGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
270	GACCTCGTGGCACAATGGTAGCACGTCTGACTctaGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
280	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTctaGATCCTTAGGTCGCTG GTTCGACTCCGGCTCGAAGGA
281	CTTTCGATAGTTCAGTTGGTAGAGCGGAGGACTctaGATCCTTAGGTCGCTG GTTCGAGTCCGGCTCGAAGGA
285	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTttaGATCCTTAGGTCGCTG GTTCGACTCCGGCTCGAAGGA
286	CTTTCGATAGTTCAGTTGGTAGAGCGGAGGACTttaGATCCTTAGGTCGCTG GTTCGAGTCCGGCTCGAAGGA

[0093] In certain embodiments, the tRNA comprises, consists essentially of, or consists of a nucleotide sequence shown in **TABLE 3**. In certain embodiments, the tRNA comprises, consists essentially of, or consists of a nucleotide sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a nucleotide sequence shown in **TABLE 3**. In certain embodiments, the tRNA comprises, consists essentially of, or consists of a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 22, 35, 36, 38, 45, 178, 180, and 187, or a nucleotide sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 22, 35, 36, 38, 45, 178, 180, and 187.

TABLE 3

SEQ ID NO	Suppressor tRNA Sequence
1	GGGCCAGTGGCGCAATGGATAACGCGTCTGACTtcaGATCAGAAGATTCCAG GTTCGACTCCTGGCTGGCTCG
3	GGGCCAGTGGCGCAATGGATAACGCGTCTGACTtcaGATCAGAAGATTCTAG GTTCGACTCCTGGCTGGCTCG

4	GGCCGCGTGGCCTAATGGATAAGGCGTCTGATTtcaGATCAGAAGATTGAGG GTTCGAGTCCCTTCGTGGTCG
6	GACCCAGTGGCCTAATGGATAAGGCATCAGCCTtcaGAGCTGGGGATTGTGG GTTCGAGTCCCATCTGGGTCG
7	GCCCCAGTGGCCTAATGGATAAGGCACTGGCCTtcaAAGCCAGGGATTGTGG GTTCGAGTCCCACCTGGGGTA
8	GCCCCAGTGGCCTAATGGATAAGGCACTGGCCTtcaAAGCCAGGGATTGTGG GTTCGAGTCCCACCTGGGGTG
9	GCCCCGGTGGCCTAATGGATAAGGCATTGGCCTtcaAAGCCAGGGATTGTGG GTTCGAGTCCCACCCGGGGTA
10	GCCCCAGTGGCCTAATGGATAAGGCATTGGCCTtcaAAGCCAGGGATTGTGG GTTCGAGTCCCATCTGGGGTG
11	GGCCGCGTGGCCTAATGGATAAGGCGTCTGACTtcaGATCAGAAGATTGCAG GTTCGAGTCCTGCCGCGGTCG
12	GACCGCGTGGCCTAATGGATAAGGCGTCTGACTtcaGATCAGAAGATTGAGG GTTCGAGTCCCTTCGTGGTCG
13	GACCACGTGGCCTAATGGATAAGGCGTCTGACTtcaGATCAGAAGATTGAGG GTTCGAATCCCTTCGTGGTTG
16	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTtcaAATTCAAAGGTTGTGG GTTCGAATCCCACCAGAGTCG
17	GGCTCCGTGGCGCAATGGATAGCGCATTGGACTtcaAGAGGCTGAAGGCATT CAAAGGTTCCGGGTTTCGAGTCCCGGCGGAGTCG
18	GGCTCCGTGGCGCAATGGATAGCGCATTGGACTtcaAATTCAAAGGTTCCGG GTTCGAGTCCCGGCGGAGTCG
22	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTtcaAATTCAAAGGTTGTGG GTTCGAGTCCCACCAGAGTCG
35	GTCTCTGTGGCGCAATGGACGAGCGCGCTGGACTtcaAATCCAGAGGTTCCG GGTTCGAGTCCCGGCAGAGATG
36	GGTCCCATGGTGTAAATGGTTAGCACTCTGGACTttaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
38	GGTTCCATGGTGTAAATGGTTAGCACTCTGGACTttaAATCCAGCGATCCGAG TTCAAATCTCGGTGGAACCT
45	GGCCCCATGGTGTAAATGGTTAGCACTCTGGACTttaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT

178	GGTCCCATGGTGTAAATGGTTAGCACTCTGGACTctaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
180	GGTTCCATGGTGTAAATGGTTAGCACTCTGGACTctaAATCCAGCGATCCGAG TTCAAATCTCGGTGGAACCT
187	GGCCCCATGGTGTAAATGGTTAGCACTCTGGACTctaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
287	GGCCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGGG TTCAAATCCCGTCGGGGTCA
288	GGCCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTACGGG TTCAAATCCCGTCGGGGTCA
289	GGCCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTCCGGG TTCAAATCCCGGCGGGGTCA
290	CGTCGGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAATTCAAAGGTT GTGGGTTCGAGTCCCAGAGTCG
291	CGTCGCCCCAGTGGCCTAATGGATAAGGCACTGGCCTTCAAAGCCAGGGATT GTGGGTTCGAGTCCCACCTGGGGTG
292	CGTCGGCTCCGTGGCGCAATGGATAGCGCATTGGACTTCAAATTCAAAGGTT CCGGGTTCGAGTCCCGGCGGAGTCG
293	CGTCGCCCCAGTGGCCTAATGGATAAGGCATTGGCCTTCAAAGCCAGGGATT GTGGGTTCGAGTCCCACCTGGGGTG
294	CGTCGGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAATTCAAAGGTT GTGGGTTCGAATCCCACCAGAGTCG
295	CGTCGGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAGCTGAGCCTAG TGTGGTCATTCAAAGGTTGTGGGTTCGAGTCCCACCAGAGTCG
296	CGTCGCCCCGGTGGCCTAATGGATAAGGCATTGGCCTTCAAAGCCAGGGATT GTGGGTTCGAGTCCCACCCGGGGTA
297	CGTCGGCTCCGTGGCGCAATGGATAGCGCATTGGACTTCAAGAGGCTGAAGG CATTCAAAGGTTCCGGGTTCGAGTCCCGGCGGAGTCG
298	CGTCGGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAGTGACGAATAG AGCAATTCAAAGGTTGTGGGTTCGAATCCCACCAGAGTCG
299	CGTCGGCCGCGTGGCCTAATGGATAAGGCGTCTGACTTCAGATCAGAAGATT GCAGGTTCGAGTCCTGCCGCGGTTCG
300	CGTCGACCGCGTGGCCTAATGGATAAGGCGTCTGACTTCAGATCAGAAGATT GAGGGTTCGAGTCCCTTCGTGGTTCG

301	CGTCGGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAGATAGTTAGAG AAATTCAAAGGTTGTGGGTTTCGAGTCCCACCAGAGTCG
302	CGTCGGTTCCATGGTGTAAATGGTGAGCACTCTGGACTCTAAATCCAGCGATC CGAGTTCGAGTCTCGGTGGAACCT
303	CGTCGGCCCCATGGTGTAAATGGTTAGCACTCTGGACTCTAAATCCAGCGATC CGAGTTCAAATCTCGGTGGGACCT
304	CGTCGGTCCCATGGTGTAAATGGTTAGCACTCTGGACTCTAAATCCAGCAATC CGAGTTCGAATCTCGGTGGGACCT
305	CGTCGGTCCCATGGTGTAAATGGTTAGCACTCTGGACTCTAAATCCAGCGATC CGAGTTCAAATCTCGGTGGGACCT
306	CGTCGGCCCCATGGTGTAAATGGTCAGCACTCTGGACTCTAAATCCAGCGATC CGAGTTCAAATCTCGGTGGGACCC
307	CGTCGGTTCCATGGTGTAAATGGTAAGCACTCTGGACTCTAAATCCAGCGATC CGAGTTCGAGTCTCGGTGGAACCT
308	CGTCGGTTCCATGGTGTAAATGGTTAGCACTCTGGACTCTAAATCCGGTAATC CGAGTTCAAATCTCGGTGGAACCT
309	CGTCGGTTCCATGGTGTAAATGGTTAGCACTCTGGACTCTAAATCCAGCGATC CGAGTTCAAGTCTCGGTGGAACCT
310	CGTCGGTTCCATGGTGTAAATGGTAAGCACTCTGGACTTTAAATCCAGCGATC CGAGTTCGAGTCTCGGTGGAACCT
311	CGTCGGCCCCATGGTGTAAATGGTTAGCACTCTGGACTTTAAATCCAGCGATC CGAGTTCAAATCTCGGTGGGACCT
312	CGTCGGTTCCATGGTGTAAATGGTGAGCACTCTGGACTTTAAATCCAGCGATC CGAGTTCGAGTCTCGGTGGAACCT
313	CGTCGGTTCCATGGTGTAAATGGTTAGCACTCTGGACTTTAAATCCAGCGATC CGAGTTCAAATCTCGGTGGAACCT
314	CGTCGGTCCCATGGTGTAAATGGTTAGCACTCTGGACTTTAAATCCAGCGATC CGAGTTCAAATCTCGGTGGGACCT
315	CGTCGGTCCCATGGTGTAAATGGTTAGCACTCTGGACTTTAAATCCAGCAATC CGAGTTCGAATCTCGGTGGGACCT
316	CGTCGGTTCCATGGTGTAAATGGTTAGCACTCTGGACTTTAAATCCGGTAATC CGAGTTCAAATCTCGGTGGAACCT
317	CGTCGGCCCCATGGTGTAAATGGTCAGCACTCTGGACTTTAAATCCAGCGATC CGAGTTCAAATCTCGGTGGGACCC

318	CGTCGGTTCCATGGTGTAAATGGTTAGCACTCTGGACTTTAAATCCAGCGATC CGAGTTCAAGTCTCGGTGGAACCT
319	CGTCGACCTCGTGGCGCAATGGTAGCGCGTCTGACTCTAGATCAGAAGGTTG CGTGTTCAAGTCACGTCGGGGTCA
320	CGTCGACCTCGTGGCGCAACGGTAGCGCGTCTGACTCTAGATCAGAAGGTTG CGTGTTCAAATCACGTCGGGGTCA
321	CGTCGGCCTCGTGGCGCAACGGTAGCGCGTCTGACTCTAGATCAGAAGGTTG CGTGTTCAAATCACGTCGGGGTCA
322	CGTCGACCTCGTGGCGCAACGGTAGCGCGTCTGACTCTAGATCAGAAGGCTG CGTGTTCGAATCACGTCGGGGTCA
323	CGTCGACCTCGTGGCGCAACGGCAGCGCGTCTGACTCTAGATCAGAAGGTTG CGTGTTCAAATCACGTCGGGGTCA
324	CGTCTCCACATGGTCTAGCGGTTAGGATTCCTGGTTCTAACCCAGGCGGCC CGGGTTCGACTCCCGGTGTGGGAA
325	CGTCTCCCATATGGTCTAGCGGTTAGGATTCCTGGTTCTAACCCAGGTGGCC CGGGTTCGACTCCCGGTATGGGAA
326	CGTCTCCCTGGTGGTCTAGTGGCTAGGATTCGGCGCTCTAACCGCCGCGGCC CGGGTTCGATTCCCGGTCAGGGAA
327	CGTCTCCCTGGTGGTCTAGTGGTTAGGATTCGGCGCTCTAACCGCCGCGGCC CGGGTTCGATTCCCGGTCAGGGAA
328	CGTCTCCCTGGTCTAGTGGCTAGGATTCGGCGCTCTAACCGCCGCGGCCCGG GTTTCGATTCCCGGCCAGGGAA
329	CGTCTCCACATGGTCTAGCGGTTAGGATTCCTGGTTCTAACCCAGGCGGCC CGGGTTCGACTCCCGGTGTGGGAA
330	CGTCTCCCATATGGTCTAGCGGTTAGGATTCCTGGTTCTAACCCAGGTGGCC CGGGTTCGACTCCCGGTATGGGAA
331	CGTCTCCCTGGTGGTCTAGTGGTTAGGATTCGGCGCTCTAACCGCCGCGGCC CGGGTTCGATTCCCGGTCAGGGAA
332	CGTCTCCCTGGTGGTCTAGTGGTTAGGATTCGGCGCTCTAACCGCCGCGGCC CGGGTTCGATTCCCGGTCAGGAAA
333	CGTCTCCCTGGTGGTCTAGTGGCTAGGATTCGGCGCTCTAACCGCCGCGGCC CGGGTTCGATTCCCGGCCAGGGAA
334	GGCCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA

335	GACCTCGTGGCGCAATGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAGTCACGTCGGGGTCA
336	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
337	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGCTGCGTG TTCGAATCACGTCGGGGTCA
338	GACCTCGTGGCGCAACGGCAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
339	GCGTTGGTGGTATAGTGGTTAGCATAGCTGCCTTCAAAGCAGTTGACCCGGG TTCGATTCCCGGCCAACGCA
340	GCGTTGGTGGTATAGTGGTGAGCATAGCTGCCTTCAAAGCAGTTGACCCGGG TTCGATTCCCGGCCAACGCA
341	GCGTTGGTGGTATAGTGGTAAGCATAGCTGCCTTCAAAGCAGTTGACCCGGG TTCGATTCCCGGCCAACGCA
342	GGCCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
343	GACCTCGTGGCGCAATGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAGTCACGTCGGGGTCA
344	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
345	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGCTGCGTG TTCGAATCACGTCGGGGTCA
346	GACCTCGTGGCGCAACGGCAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
347	GGCCTCATGGTGCAACAGTAGTGTGTCTGACTTCAGATCAGAAGGTTGTATG TTCAAATCACGTAGGGGTCA
348	GGCCTCGTGGCGCAACGGTAGCGCGTCTGACTCTAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
349	GACCTCGTGGCGCAATGGTAGCGCGTCTGACTCTAGATCAGAAGGTTGCGTG TTCAAGTCACGTCGGGGTCA
350	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTCTAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
351	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTCTAGATCAGAAGGCTGCGTG TTCGAATCACGTCGGGGTCA

352	GACCTCGTGGCGCAACGGCAGCGCGTCTGACTCTAGATCAGAAGGTTGCGTG TTCAAATCACGTCTGGGGTCA
353	GGCCTCATGGTGCAACAGTAGTGTGTCTGACTCTAGATCAGAAGGTTGTATG TTCAAATCACGTAGGGGTCA
354	GCATTGGTGGTTCAGTGGTAGAATTCTCGCCTTCAACGCGGGAGACCCGGGT TCAATTCCCGGCCAATGCA
355	GCGCCGCTGGTGTAGTGGTATCATGCAAGATTTCAATTCTTGCGACCCGGGT TCGATTCCCGGGCGGCGCA
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761	CCCCGGCTGGCTCAGTCAGTAGATCATGAGACTTTAAATCTCAGGGTCGTGG GTTACGCCCCACACTGGGCG
762	GCGCTAGTCAGTAGAGCATGAGACTTTAAATCTCAGGGTCGTGGGTTCGAGC CCCACATCGGGCG
763	GCCTGGATAGCTCAGTTGGTAGAGCATCAGACTTTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTTCAGGCA
764	GCCAGGATAGTTTCAGGTGGTAGAGCATCAGACTTTAAACCTGAGGGTTCAGG GTTCAAGTCTCTGTTTGGGCG
765	ACCCAGATAGCTCAGTCAGTAGAGCATCAGACTTTAAATCTGAGGGTCCAAG GTTTCATGTCCCTTTTTGGGTG
766	ACCTGGGTAGCTTAGTTGGTAGAGCATTGGACTTTAAATTTGAGGGCCCAGG TTTCAAGTCCCTGTTTGGGTG
767	GCCTGGGTAGCTCAGTCGGTAGAGCTATCAGACTTTAAGCCTGAGGATTCAG GGTTCAATCCCTTGCTGGGGCG

768	GATAGCTCAGTTGATAGAGCATCAGACTTTAAATCTGAGGGTCCAGGGTTCA TGTCCTGT
769	GTTGGGGTAACTCAGTTGGTAGAGTAGCAGACTTTACATCTGAGGGTCCAGG GTTTAAGTCCATGTCCAGGCA
770	GCCTGGATAGCTCAGTTGGTAGAGCATCAGACTTTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTCCAGGCG
771	GCCTGGATAGCTCAGTCGGTAGAGCATCAGACTTTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTCCAGGCG
772	GCCCGGATAGCTCAGTCGGTAGAGCATCAGACTTTAAATCTGAGGGTCCGGG GTTCAAGTCCCTGTTCCGGGCG
773	GCCTGGGTAGCTCAGTCGGTAGAGCATCAGACTTTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTCCAGGCG
774	GCCTGGATAGCTCAGTTGGTAGAACATCAGACTTTAAATCTGACGGTGCAGG GTTCAAGTCCCTGTTCCAGGCG
775	GCCCGGAGAGCTCAGTGGGTAGAGCATCAGACTTTAAATCTGAGGGTCCAGG GTTCAAGTCCTCGTTCCGGGCA
776	ACCTGGGTAGCTCAGTAGGTAGAACATCAGACTTTAAATCTGAGGGTCTAGG GTTCAAGTCCCTGTCCAGGCG
777	GCCTGGATAGCTCCTTCGGTAGAGCATCATCAGACTTTAAATGTGAGGGTCC AGGGTTCAAGTTCCTGTTTGGGCG
778	GCCCAGCTAGCTCAGTCGGTAGAGCATAAGACTCTAAATCTCAGGGTTGTGG ATTCGTGCCCCATGCTGGGTG
779	CTGCAGCTAGCTCAGTCGGTAGAGCATGAGACTCTAAATCTCAGGGTCATGG GTTTCGTGCCCCATGTTGGG
780	CCAGCATGTCTCAGTCGGTATAGTGTGAGACTCTAAATCTCAGGGTCGTGG TTCAAGCCCCACATTGGG
781	GTCTAGCTAGATCAGTTGGTAGAGCATAAGACTCTAAATCTCAGGGTCATGG GTTTGAGCCCTACGTTGGGCG
782	GCCCAGCTAGCTCAGCCGGTAGAGCACAAGACTCTAAATCTCAGGGTCGTGG GTTTGAGCCCTGTGTTGAGCA
783	CCGAATAGCTTAGTTGATGAAGCGTGAGACTCTAAATCTCAGGGTAGTGGGT TCAAGCCCCACATTGGA
784	GCCTGGCTACCTCAGTTGGTAGAGCATGGGACTCTAAATCCCAGAGTCAGTG GGTTCAAGCCTCACATTGAGTG

785	GCCCGGCTAGCTCAGTCGGTAGAGCATGAGACCCTAAATCTCAGGGTCGTGG GTTCGAGCCCCACGTTGGGCG
786	GCCCGGCTAGCTCAGTCGGTAGAGCATGGGACTCTAAATCTCAGGGTCGTGG GTTCGAGCCCCACGTTGGGCG
787	GCCCGGCTAGCTCAGTCGATAGAGCATGAGACTCTAAATCTCAGGGTCGTGG GTTCGAGCCGCACGTTGGGCG
788	GCCCAGCTAGCTCAGTCGGTAGAGCATGAGACTCTAAATCTCAGGGTCATGG GTTTGAGCCCCACGTTTGGTG
789	GCCTGGCTAGCTCAGTCGGCAAAGCATGAGACTCTAAATCTCAGGGTCGTGG GCTCGAGCTCCATGTTGGGCG
790	GCCCGACTACCTCAGTCGGTGGAGCATGGGACTCTACATCCCAGGGTTGTGG GTTCGAGCCCCACATTGGGCA
791	CCCCGGCTGGCTCAGTCAGTAGATCATGAGACTCTAAATCTCAGGGTCGTGG GTTACGCCCCACACTGGGCG
792	GCGCTAGTCAGTAGAGCATGAGACTCTAAATCTCAGGGTCGTGGGTTCGAGC CCCACATCGGGCG
793	GCCTGGATAGCTCAGTTGGTAGAGCATCAGACTCTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTTCAGGCA
794	GCCAGGATAGTTCAGGTGGTAGAGCATCAGACTCTAAACCTGAGGGTTCAGG GTTCAAGTCTCTGTTTGGGCG
795	ACCCAGATAGCTCAGTCAGTAGAGCATCAGACTCTAAATCTGAGGGTCCAAG GTTCAATGTCCCTTTTTGGGTG
796	ACCTGGGTAGCTTAGTTGGTAGAGCATTTGGACTCTAAATTTGAGGGCCCAGG TTTCAAGTCCCTGTTTGGGTG
797	GCCTGGGTAGCTCAGTCGGTAGAGCTATCAGACTCTAAGCCTGAGGATTCAG GGTTCAATCCCTTGCTGGGGCG
798	GATAGCTCAGTTGATAGAGCATCAGACTCTAAATCTGAGGGTCCAGGGTTCA TGTCCCTGTT
799	GTTGGGGTAACTCAGTTGGTAGAGTAGCAGACTCTACATCTGAGGGTCCAGG GTTTAAGTCCATGTCCAGGCA
800	GCCTGGATAGCTCAGTTGGTAGAGCATCAGACTCTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTTCAGGCG
801	GCCTGGATAGCTCAGTCGGTAGAGCATCAGACTCTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTTCAGGCG

802	GCCCGGATAGCTCAGTCGGTAGAGCATCAGACTCTAAATCTGAGGGTCCGGG GTTCAAGTCCCTGTTCTGGGCG
803	GCCTGGGTAGCTCAGTCGGTAGAGCATCAGACTCTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTCCAGGCG
804	GCCTGGATAGCTCAGTTGGTAGAACATCAGACTCTAAATCTGACGGTGCAGG GTTCAAGTCCCTGTTCTAGGCG
805	GCCCGGAGAGCTCAGTGGGTAGAGCATCAGACTCTAAATCTGAGGGTCCAGG GTTCAAGTCCCTCGTTCTGGGCA
806	ACCTGGGTAGCTCAGTAGGTAGAACATCAGACTCTAAATCTGAGGGTCTAGG GTTCAAGTCCCTGTCCAGGCG
807	GCCTGGATAGCTCCTTCGGTAGAGCATCATCAGACTCTAAATGTGAGGGTCC AGGGTTCAAGTTCCTGTTTGGGCG
808	GGCAGAATGGTGCAGCGGTTAGCACCCAGGCTCTTCAGCCAGCTGTTGCCT GGGCTCAAATCCCAGCTCTGCCA
809	GGCTGTATAGCTCAGTGGTAGAGCATTTGACTTCAGAATCCTATACTCAGGG GAAGGAGAACTGGGGGTTTCTCAGTGGGTCAAAGGACTTGTAGTGGTAAATC AAAAGCAACTCTATAAGCTATGTAACAACTTTAAAGTCATATGTAGCTGGG TTCAAATCCTGTTTCTGCCA
810	GGCTGTATAGCTCAGTGGTAGAGCATTTGACTTCAGCTTTAAAGTCATATGT AGCTGGGTTCAAATCCTGTTTCTGCCA
811	GGGGGCATAGCTCAGTGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
812	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCCT
813	GGGGGTATAGCTTAGCGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCCGG TTCAAATCCGGGTGCCCCCT
814	GGGGGTATAGCTTAGGGGTAGAGCATTTGACTTCAGATCAAAAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
815	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCCAG TTCAAATCTGGGTGCCCCCT
816	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTTCAGATCAAGAAGTCCCCGG TTCAAATCCGGGTGCCCCCT
817	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTTCAGATCAAGAGGTCTCTGG TTCAAATCCAGGTGCCCCCT

818	GGGGGTATAGCTCAGGGGTAGAGCACTTGACTTCAGATCAAGAAGTCCTTGG TTCAAATCCAGGTGCCCCCT
819	GGGGATATAGCTCAGGGGTAGAGCATTGACTTCAGATCAAGAGGTCCCCGG TTCAAATCCGGGTGCCCCCCT
820	GGGGGTATAGTTCAGGGGTAGAGCATTGACTTCAGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
821	GGGGGTATAGCTCAGGGGTAGAGCATTGACTTCAAATCAAGAGGTCCCTGA TTCAAATCCAGGTGCCCCCT
822	GGGCGTATAGCTCAGGGGTAGAGCATTGACTTCAGATCAAGAGGTCCCCAG TTCAAATCTGGGTGCCCCCT
823	GGGGGTATAGCTCACAGGTAGAGCATTGACTTCAGATCAAGAGGTCCCCGG TTCAAATCTGGGTGCCCCCT
824	GGGCGTATAGCTCAGGGGTAGAGCATTGACTTCAGATCAAGAGGTCCCCAG TTCAAATCTGGGTGCCCA
825	GGGGGTATAGCTCACAGGTAGAGCATTGACTTCAGATCAAGAGGTCCCCGG TTCAAATCCGGTTACTCCCT
826	GGGGGTAGGGCTCAGGGATAGAGCATTGACTTCAGATCAAGAGGTCCCCGG TTCGAATCTAGGTGCCCCCT
827	GGTATATCTCAGGGGGCAGAGCATTGACTTCAGATCAAGAGGTCCCCGGTT GAAATCCGGGTGCT
828	GGGGGTATAGCTCAGGGGTAGAGCACTTGACTTCAGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
829	GGGGGTATAGCTCAGTGGTAGAGCATTGACTTCAGATCAAGAGGTCCCTGG TTCAAATCCGGGTGCCCCCT
830	GGGGGTATAGCTCAGTGGGTAGAGCATTGACTTCAGATCAAGAGGTCCCCG GTTCAAATCCGGGTGCCCCCT
831	GGGGGTGTAGCTCAGTGGTAGAGCATTGACTTCAGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
832	GGGGGTATAGCTCAGGGGTAGAGCATTGACTTCAGATCAAGAGGTCCCCGG TTCAAATCCGGGTGCCCCCT
833	GGGGGTATAGCTCAGGGGTAGAGCATTGACTTCAGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
834	GACCTCGTGGCGCAATGGTAGCGGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA

835	GACCTCGTGGCACAATGGTAGCACGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
836	GAAGCGGTGGCTCAATGGTAGAGCTTTCGACTTCAATTAAATCTTGGAAATT CCACGGAATAAGATTGCAATCGAAGGGTTGCAGGTTCAATTCCTGTCCGTTT CA
837	GAAGCGGTGGCTCAATGGTAGAGCTTTCGACTTCAAATCGAAGGGTTGCAGG TTCAATTCCTGTCCGTTTCA
838	GGCCTCATGGTGCAACAGTAGTGTGTCTGACTTCAGATCAGAAGGTTGTATG TTCAAATCACATAGGGGTCA
839	GACCTCGTGGTGAAATGGTAGCATGTTTGACTTCAAATCAGGAGGTTGTGTG TTCAAGTCACATCAGGGTCA
840	GACCTTGTGGCGCAATGGTAGCATGTTTGACTTCAAATCAGGAGGTTGTGTG TTCAAGTCACATCAGGGTCA
841	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGCTGCGTG TTCGAATCACGCCGGGGTCA
842	GACCTTGTGGCTCAATGGTAGCGCATCTGACTTCAGATCAGGAGGTTGCACG TTCAAATCATGCCGGGGTCA
843	GACCTTGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
844	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTA TTCAAATCACGTCGGGGTCA
845	GACCTCGTGGCGCAACGGCAGCGCGTCTGACTTCACATTAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
846	GACCTCATGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACATCGGGGTCA
847	GACCTCGTGGTGCAACGGTAGCGCGTATGATTTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
848	GACCTCGTAGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
849	AGGGGTATAGCTCAATTGGCAGAGCGTCGGTCTTCAAACCGAAGGTTGTAG GTTTCGATTCCTACTGCCCCTGCCA
850	GACCTCATGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA

851	GACCTCGTGGCGCAACGGTAGCGCGTCTAACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
852	ACGGGAGTAGCTCAGTTGGTAGAGCACCGGTCTTCAAACCGGGTGTCGGGA GTTCGAGCCTCTCCTCCCGTG
853	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCATG TTCAAATCACGTCGGGGTCA
854	GACTCCGTGGCGCAACGGTAGCGCGTCCGACTTCAGATCGGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
855	GACTCCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
856	GGCCTCGTGGCGCAACGGTAGCGCGTCTGACTCCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
857	GGCCTCGTGGCGCAACGGTAGCACGTCTGACTCCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
858	CGGCCTCGTGGCGCAACGGTAGCACGTCTGACTTCAGATCAGAAGGTTGCGT GTTCAAATCACGTCGGGGTCA
859	GGCCTCGTCGCGCAACGGTAGCGCGTCTGACTCCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
860	GGCCTCGTCGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
861	GGCCTCGTCGCGCAACGGTAGCACGTCTGACTCCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
862	GGCCTCGTCGCGCAACGGTAGCACGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA

[0094] In certain embodiments, the tRNA comprises, consists essentially of, or consists of a nucleotide sequence shown in any one of **TABLEs 8-10**. In certain embodiments, the tRNA comprises, consists essentially of, or consists of a nucleotide sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a nucleotide sequence in any one of **TABLEs 8-10**.

[0095] In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 6. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 7. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID

NO: 8. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 9. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 11. In certain
5 embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 16. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 17. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 18. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 19. In certain embodiments, the tRNA comprises, consists essentially
10 of, or consists of the nucleotide sequence of SEQ ID NO: 20. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 21. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 22. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 35. In certain embodiments, the tRNA
15 comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 36. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 37. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 38. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 39. In
20 certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 40. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 44. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 45. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide
25 sequence of SEQ ID NO: 178. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 179. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 180. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 181. In certain embodiments, the tRNA comprises, consists
30 essentially of, or consists of the nucleotide sequence of SEQ ID NO: 182. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 186. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 187.

[0096] In certain embodiments, the tRNA may comprise one or more mutations (*e.g.*, nucleotide substitutions, deletions, or insertions) relative to a reference tRNA sequence (*e.g.*, a tRNA disclosed herein). In certain embodiments, the tRNA may comprise, consist, or consist essentially of, a single mutation, or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more than 15 mutations. It is contemplated that the tRNA may comprise, consist, or consist essentially 1-15, 1-10, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-15, 2-10, 2-7, 2-6, 2-5, 2-4, 2-3, 3-15, 3-10, 3-7, 3-6, 3-5, or 3-4 mutations.

[0097] Sequence identity may be determined in various ways that are within the skill in the art, *e.g.*, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. BLAST (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs blastp, blastn, blastx, tblastn and tblastx (Karlin *et al.*, (1990) PROC. NATL. ACAD. SCI. USA 87:2264-2268; Altschul (1993) J. MOL. EVOL. 36, 290-300; Altschul *et al.*, (1997) NUCLEIC ACIDS RES. 25:3389-3402) are tailored for sequence similarity searching. For a discussion of basic issues in searching sequence databases see Altschul *et al.* (1994) NATURE GENETICS 6:119-129. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. The search parameters for histogram, descriptions, alignments, expect (*i.e.*, the statistical significance threshold for reporting matches against database sequences), cutoff, matrix and filter are at the default settings. The default scoring matrix used by blastp, blastx, tblastn, and tblastx is the BLOSUM62 matrix (Henikoff *et al.*, (1992) PROC. NATL. ACAD. SCI. USA 89:10915-10919). Four blastn parameters may be adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (generates word hits at every wink.sup.th position along the query); and gapw=16 (sets the window width within which gapped alignments are generated). The equivalent Blastp parameter settings may be Q=9; R=2; wink=1; and gapw=32. Searches may also be conducted using the NCBI (National Center for Biotechnology Information) BLAST Advanced Option parameter (*e.g.*: -G, Cost to open gap [Integer]: default = 5 for nucleotides/ 11 for proteins; -E, Cost to extend gap [Integer]: default = 2 for nucleotides/ 1 for proteins; -q, Penalty for nucleotide mismatch [Integer]: default = -3; -r, reward for nucleotide match [Integer]: default = 1; -e, expect value [Real]: default = 10; -W, wordsize [Integer]: default = 11 for nucleotides/ 28 for megablast/ 3 for proteins; -y, Dropoff (X) for blast extensions in bits: default = 20 for blastn/ 7 for others; -X, X dropoff value for gapped alignment (in bits): default = 15 for all programs, not applicable to blastn; and -Z, final X dropoff value for gapped alignment (in bits): 50 for blastn, 25 for others). ClustalW for pairwise protein alignments may also be

used (default parameters may include, *e.g.*, Blosum62 matrix and Gap Opening Penalty = 10 and Gap Extension Penalty = 0.1). A Bestfit comparison between sequences, available in the GCG package version 10.0, uses DNA parameters GAP=50 (gap creation penalty) and LEN=3 (gap extension penalty) and the equivalent settings in protein comparisons are GAP=8 and LEN=2.

- 5 [0098] It is contemplated that a tRNA may comprise one or more modifications. Exemplary modified tRNAs include: acylated tRNA; alkylated tRNA; a tRNA containing one or more bases other than adenine, cytosine, guanine, or uracil; a tRNA covalently modified by the attachment of a specific ligand or antigenic, fluorescent, affinity, reactive, spectral, or other probe moiety; a tRNA containing one or more ribose moieties that are methylated or otherwise modified; aa-
- 10 tRNAs that are aminoacylated with an amino acid other than the 20 natural amino acids, including non-natural amino acids that function as a carrier for reagents, specific ligands, or as an antigenic, fluorescent, reactive, affinity, spectral, or other probe; or any combination of these compositions. Exemplary modified tRNA molecules are described in Soll *et al.* (1995) "tRNA: Structure, Biosynthesis, and Function," ASM Press; El Yacoubi *et al.* (2012) ANNU. REV. GENET. 46:69-95; Grosjean *et al.* (1998) "Modification and Editing of RNA." ASM Press;
- 15 Hendrickson *et al.* (2004) ANNU. REV. BIOCHEM. 73:147-176, 2004; Ibba *et al.* (2000) ANNU. REV. BIOCHEM. 69:617-650; Johnson *et al.* (1995) COLD SPRING HARBOR SYMP. QUANT. BIOL. 60:71-82; Johnson *et al.* (1982) J. MOL. BIOL. 156:113-140; Crowley *et al.* (1994) CELL 78:61-71; Beier *et al.* (2001) NUCLEIC ACIDS RES. 29:4767-4782; Torres *et al.* (2014) TRENDS MOL. MED. 20:306-314; Bjork *et al.* (1987) ANNU. REV. BIOCHEM. 56:263-287; Schaffrath *et al.* (2017) RNA BIOL. 14(9):1209-1222; and Johansson *et al.* (2008) MOL. CELL. BIOL. 28(10):3301-12.

- [0099] In certain embodiments, a tRNA comprises a naturally occurring nucleotide modification. Naturally occurring tRNAs contain a wide variety of post-transcriptionally
- 25 modified nucleotides, which are described, for example, in Machnicka *et al.* (2014) RNA BIOLOGY 11(12): 1619-1629, and include one or more of the residues as shown in **FIGURE 2B**. In certain embodiments, the tRNA comprises one or more of the residues selected from the group consisting of: 2'-O-methylguanosine or G at position 0; pseudouridine or U at position 1; 2'-O-methyladenosine, A, 2'-O-methyluridine, U, 2'-O-methylcytidine, C, 2'-O-
- 30 methylguanosine, or G at position 4; N2-methylguanosine or G at position 6; N2-methylguanosine or G at position 7; 1-methyladenosine, A, 1-methylguanosine, G, or a modified G at position 9; N2-methylguanosine or G at position 10; N4-acetylcytidine or C at position 12; pseudouridine, U, 2'-O-methylcytidine, or C at position 13; 1-methyladenosine, A, or a modified A at position 14; dihydrouridine (D) or U at position 16; D or U at position 17; 2'-O-

methylguanosine or G at position 18; 3-(3-amino-3-carboxypropyl)uridine, D, or U at position 20; 3-(3-amino-3-carboxypropyl)uridine, D, pseudouridine, U, or a modified U at position 20a; D, pseudouridine, or U at position 20b; pseudouridine or U at position 25; pseudouridine, U, N2,N2-dimethylguanosine, N2-methylguanosine, G, or a modified G at position 26;

5 pseudouridine, U, N2,N2-dimethylguanosine, or G at position 27; pseudouridine or U at position 28; pseudouridine or U at position 30; pseudouridine or U at position 31; 2'-O-methylpseudouridine, 2'-O-methyluridine, pseudouridine, U, 2'-O-methylcytidine, 3-methylcytidine, C, or a modified C at position 32; inosine, A, 2-thiouridine, 2'-O-methyluridine, 5-(carboxyhydroxymethyl)uridine methyl ester, 5-carbamoylmethyluridine, 5-

10 carboxymethylaminomethyl-2'-O-methyluridine, 5-methoxycarbonylmethyl-2-thiouridine, 5-methoxycarbonylmethyluridine, pseudouridine, U, a modified U, 2'-O-methylcytidine, 5-formyl-2'-O-methylcytidine, 5-methylcytidine, C, a modified C, queuosine, mannosyl-queuosine, galactosyl-queuosine, 2'-O-methylguanosine, or G at position 34; pseudouridine or U at position 35; pseudouridine, U, or a modified U at position 36; 1-methylinosine, 2-methylthio-N6-

15 threonylcarbamoyladenosine, N6-isopentenyladenosine, N6-methyl-N6-threonylcarbamoyladenosine, N6-threonylcarbamoyladenosine, A, a modified A, 1-methylguanosine, peroxywybutosine, wybutosine, G, or a modified G at position 37; pseudouridine, U, 5-methylcytidine, C, or a modified C at position 38; 1-methylpseudouridine, 2'-O-methylpseudouridine, 2'-O-methyluridine, pseudouridine, U, 2'-O-methylguanosine, or G at

20 position 39; pseudouridine, U, 5-methylcytidine, or C at position 40; 2'-O-methyluridine, U, or a modified U at position 44; pseudouridine or U at position e11; pseudouridine or U at position e12; pseudouridine or U at position e14; 3-methylcytidine or C at position e2; 7-methylguanosine or G at position 46; D, U, or a modified U at position 47; D, U, 5-methylcytidine, C, or a modified C at position 48; A, a modified A, 5-methylcytidine, C, or a

25 modified C at position 49; pseudouridine, U, 5-methylcytidine, or C at position 50; 5,2'-O-dimethyluridine, 5-methyluridine, pseudouridine, or U at position 54; pseudouridine or U at position 55; 1-methyladenosine, A, or a modified A at position 58; 2'-O-ribosyladenosine (phosphate), A, 2'-O-ribosylguanosine (phosphate), G, or a modified G at position 64; pseudouridine or U at position 65; pseudouridine, U, N2-methylguanosine, or G at position 67;

30 pseudouridine or U at position 68; and, pseudouridine, U, 5-methylcytidine, or C at position 72. A, C, G, and U, refer to unmodified adenine, cytosine, guanine, and uracil, respectively. The numbering of the residues is based on the tRNA numbering system described in Steinberg *et al.*, (1993) NUCLEIC ACIDS RES. 21:3011-15.

[00100] In certain embodiments, the 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.

5 **II. Methods Of Making tRNAs**

[00101] It is contemplated the tRNA molecules (*e.g.*, suppressor tRNAs) useful in the practice of the invention can be produced by methods known in the art, including extracellular production by synthetic chemical methods, intracellular production by recombinant DNA methods, or purification from natural sources.

10 [00102] For example, DNA molecules encoding tRNAs can be synthesized chemically or by recombinant DNA methodologies. For example, the sequences of the tRNAs can be synthesized or cloned from libraries by conventional hybridization techniques or polymerase chain reaction (PCR) techniques, using the appropriate synthetic nucleic acid primers. The resulting DNA molecules encoding the tRNAs can be ligated to other appropriate nucleotide sequences,
15 including, for example, expression control sequences to produce conventional gene expression constructs (*i.e.*, expression vectors) encoding the tRNAs. Production of defined gene constructs is within routine skill in the art. Nucleic acids encoding desired tRNAs can be incorporated (ligated) into expression vectors, such as the expression vectors described in the following section, which can be introduced into host cells through conventional transfection or
20 transformation techniques. Exemplary host cells are *E. coli* cells, Chinese hamster ovary (CHO) cells, human embryonic kidney 293 (HEK 293) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), and myeloma cells. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the tRNAs. Specific expression and purification conditions will
25 vary depending upon the expression system employed.

[00103] Alternatively, tRNAs can be chemically synthesized or purified from natural sources by methods known in art. When a tRNA is aminoacylated prior to introduction into the cell or administration to the subject, the tRNA may be aminoacylated with a desired amino acid by any method known in the art, including chemical or enzymatic aminoacylation.

30 **III. Expression Vectors**

[00104] The tRNAs of interest may be expressed in a cell of interest by incorporating a gene encoding a tRNA of interest into an appropriate expression vector. As used herein, "expression vector" refers to a vector comprising a recombinant polynucleotide comprising

expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis- acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (*e.g.*, naked or contained in liposomes), retrotransposons (*e.g.* piggyback, sleeping beauty), and viruses (*e.g.*, lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide of interest.

[00105] In certain embodiments, the expression vector is a viral vector. The term "virus" is used herein to refer to an obligate intracellular parasite having no protein-synthesizing or energy-generating mechanism. Exemplary viral vectors include retroviral vectors (*e.g.*, lentiviral vectors), adenoviral vectors, adeno-associated viral vectors, herpesviruses vectors, epstein-barr virus (EBV) vectors, polyomavirus vectors (*e.g.*, simian vacuolating virus 40 (SV40) vectors), poxvirus vectors, and pseudotype virus vectors.

[00106] The virus may be a RNA virus (having a genome that is composed of RNA) or a DNA virus (having a genome composed of DNA). In certain embodiments, the viral vector is a DNA virus vector. Exemplary DNA viruses include parvoviruses (*e.g.*, adeno-associated viruses), adenoviruses, asfarviruses, herpesviruses (*e.g.*, herpes simplex virus 1 and 2 (HSV-1 and HSV-2), epstein-barr virus (EBV), cytomegalovirus (CMV)), papillomoviruses (*e.g.*, HPV), polyomaviruses (*e.g.*, simian vacuolating virus 40 (SV40)), and poxviruses (*e.g.*, vaccinia virus, cowpox virus, smallpox virus, fowlpox virus, sheeppox virus, myxoma virus). In certain embodiments, the viral vector is a RNA virus vector. Exemplary RNA viruses include bunyaviruses (*e.g.*, hantavirus), coronaviruses, flaviviruses (*e.g.*, yellow fever virus, west nile virus, dengue virus), hepatitis viruses (*e.g.*, hepatitis A virus, hepatitis C virus, hepatitis E virus), influenza viruses (*e.g.*, influenza virus type A, influenza virus type B, influenza virus type C), measles virus, mumps virus, noroviruses (*e.g.*, Norwalk virus), poliovirus, respiratory syncytial virus (RSV), retroviruses (*e.g.*, human immunodeficiency virus-1 (HIV-1)) and toroviruses.

[00107] In certain embodiments, the expression vector comprises a regulatory sequence or promoter operably linked to the nucleotide sequence encoding the tRNA. The term "operably linked" refers to a linkage of polynucleotide elements in a functional relationship. A nucleic acid sequence is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a gene if it affects the transcription of the gene. Operably linked nucleotide sequences are typically contiguous. However, as enhancers generally function when separated from the promoter by

several kilobases and intronic sequences may be of variable lengths, some polynucleotide elements may be operably linked but not directly flanked and may even function *in trans* from a different allele or chromosome.

[00108] tRNA genes preferably have strong promoters that are active in a variety of cell types. The promoters for eukaryotic tRNA genes typically are present within the structural sequences encoding the tRNA molecule itself. Although there are elements which regulate transcriptional activity within the 5' upstream region, the length of an active transcriptional unit may be considerably less than 500 base pairs.

[00109] Additional exemplary promoters which may be employed include, but are not limited to, the retroviral LTR, the SV40 promoter, the human cytomegalovirus (CMV) promoter, the U6 promoter, or any other promoter (*e.g.*, cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and β -actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, TK promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

[00110] In certain embodiments, an expression vector comprises a tRNA coding sequence that encodes a tRNA that comprises, consists essentially of, or consists of a nucleotide sequence shown in **TABLE 2** or **TABLE 3**. In certain embodiments, an expression vector comprises a tRNA coding sequence that encodes a tRNA that comprises, consists essentially of, or consists of a nucleotide sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% to a nucleotide sequence shown in **TABLE 2** or **TABLE 3**.

[00111] In certain embodiments, in addition to a tRNA coding sequence, the expression vector comprises a nucleotide sequence corresponding to a genomic DNA sequence flanking a wild-type tRNA gene (*i.e.*, a DNA sequence from the same genome as a wild-type tRNA gene and which is 5' or 3' to the wild-type tRNA gene in the genome, *e.g.*, immediately 5' or 3' to the wild-type tRNA gene in the genome). In certain embodiments, in addition to a tRNA coding sequence, the expression vector comprises a nucleotide sequence corresponding to an exogenous promoter.

[00112] In certain embodiments, the expression vector comprises a nucleotide sequence shown in **TABLE 4**. In certain embodiments, the expression vector comprises a nucleotide sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a nucleotide sequence shown in **TABLE 4**. In certain embodiments, in the expression vector, the nucleotide sequence set forth in **TABLE 4** is

operably linked to the nucleotide sequence encoding the tRNA. In certain embodiments, in the expression vector, the nucleotide sequence set forth in **TABLE 4** is 5' or 3' (*e.g.*, immediately 5' or immediately 3') to the nucleotide sequence encoding the tRNA. In certain embodiments, the expression vector comprises a nucleotide sequence selected from SEQ ID NOs: 869-888, or a
5 nucleotide sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a sequence selected from SEQ ID NOs: 869-888.

[00113] In certain embodiments, the expression vector is an expression vector described in Example 8 or Example 9 herein.

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TABLE 4

SEQ ID NO	Location (Relative to tRNA)	Nucleotide Sequence
26	5'	CTACCCAGAGGCAGGCGGGAGACTCCCCCGAGCGTCCAATAAGAGC GCCGCCAATGGAGCCGCCCCGCCGCGGGGGTGCAGAGGGACTTCCG GGTGAGGTCCTCCGCTACTTCCCTCCCCACGGAAAAGATAGACCAG TCTGACGCGAGCCTGAAGGCGGCTACACGCTTTAAGCTAAGTAAAG GCACCTTCTCGCTGGC
27	3'	ACTTGTATGTTGTTTTTATCTGTCAGTTTGTTAATCCCAAGATTCC CTTTGGAAATAAAGCGAAATTGACCGTAGTGGTTATGACCAACTTC TAGTCTAAACTTAATTCTTGGA ACTCAAGGATCTGAGCAAACA ACT GTCAGGGTGACACATTGCTTAAACGGTGACAGCGGTCGAGAGCCTT GTCCCGGATGGAGAGT
32	3'	ACTTGTATGTTGTTTTTATCTGTCAGTTTGTTAATCCCAAGATTCC CTTTGGAAATAAAGCGAAATTGACCGTAGTGGTTATGACCAACTTC TAGTCTAAACTT
33	5'	GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACA AGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAA GATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGG TAGTTTGCAGTTTTTAAAATTATGTTTTTAAAATGGACTATCATATGC TTACCGTAACTTGAAAGTATTTGATTTCTTGGCTTTATATATCTT GTGGAAAGGACGGGCGGAGGAAGGCACCTTCTCGCTGGC
34	3'	ACTTGTATGTTGTTTTTATCTGTCAGTTTGTTAATCCCAAGATTCC

173	5'	GATCACCGGAAGAGGTGACAGACACCTCGGGGCCCATGAACGTTTG GAATTCGTAAGGACATGAGAATCTCGGTGGTTCCGTGTCTGCCCCG CATCGCGGCCACCGGCCACGGGCCCAAGCCAAGTGTAGCGAAGCTT AGAAAAGGTTGCCCAACGTCATGTGGCTTGAGAAGGCTGCCGGGCG CCTTAAGCCGCCAGCA
174	3'	CACTGAACCTTTTTTTGGCCTTAGAATCCCTGTTTTGGGGCCTGCA GGAAGTAGCAACCAACCCGAGCCTCCGCAGGGAATGCACTGACCTG TAGAATGGACGTTTCACTTCCCTCCCTGTGTCTCAACACGATTACA TTTCAGGAACAGCCTGGGCTGGGAGGCACTGCGCACGCGCGCCGAG TCGGGCGGAAAAATAA
869	5'	CCAAAACATCTTTTACTGTAGTATCTACTTACCATACTACCCAAGA ATGGCACACTGCTCACATCTTCAAAAGCTTAAACCAAGAGCACTAC ACAGGTGC
870	5'	TGTGTGTCGGGGCCGGTACCCTGCTTCCGGTTCCCGCACGCATTCC CGGATTGCAGTGCGGACCCCTTCTGTAAGCGCGCGATAAAGCGCGG TTTTGGAA
871	5'	TCATGTCATATAAGTAGAACCATAACAATATATATATAAAATCCAGG TTAATAGCCAATCTTACAACATTTCTCATATTTTTTGCAGTTGCTA AGCCATGG
872	5'	ACATTACAATACATATCAACATATCACCATAATTAAATTGCAAGTC TTCGTCAAAAGCAAGCCTTAAAGGAGTATCCCAAAAACACATTTTC CCCAGAAG
873	5'	AGACCTTTAGAGCGTGGTTAAACCCATATGTTGGGATTTATGCTGC TTTTATGGTAGCAATACCCTATATTAAGATTTGAAGTAGACCCGGA AAGTTAGT
874	5'	G TTCATGAAAGAATAAATAAATGTTTAAAAAAAAAAAAAACTGAGG TAAATTTCTATATTCTTTCATAAAAGCAGTTTAAAGACGAACGTTT TTCGAGGT
875	5'	GCTGGGTCTCGGTGACACTGACGACGGGAGGCGCGGTTCGGAAGAGC GCGGGGCCGTCGCCTCTGGCTTAACATAGCAGATGCGCTGAGACTC CAACAGGT
876	5'	CAGTGGCGGCGAAACTCTCTGCGTTCTGGAGGGAGGGTGCGGGCA GGAGGAGGTAGAGGATGCCTTGTAAGCGGAGCAAAAACAAGGTTCA ACGTCTGC

877	5'	CAAATCACTTGCCTCTCGGCGCGAGACCGCGATGCGCGGGGGCGGG AGCGTGATGATGGCATCGCGTAAGGAGAGGGTGTGAGAAGCCGGAT CCTGTGGT
878	5'	CCCTGTGTCCGAAGAGGTCTGCGTTGCGACTTACGTGGTAGTGCTT GGAAGGTGCGGAGTAGATGAGAGATAAGTGAATGTGGACAAACCTG TCACGTAG
879	5'	GAGCGGAGCTCAGAGGGTGCGCGCTCCGCCCTTTCGCGGGCCTGGC ATGAGCGCAGTGGTTGTTACACTAAAGTGTCTCCGCCTGTCGAATA TTCTCGTG
880	5'	GTGTCACTGGTTTCAAATCAACCTCAATTTTTTTGGAGACGTGAGT GCTGAGCATTTTTTCTTCAGTGAAGTGACTTGGCAGCCAAAATCGC CAACGCCC
881	5'	TCCTGGCATGTCCCGCCCAAGTCCCTTAGCCCCGCTCCCCAACCT GCCCCATTCCCCTCTAGTACCCGTAAGCTACAAGACGCCGCCGTT CGTCGGGT
882	5'	TGCTCAGTCGTCCTGCCGGGCGGGCCCTGAGGTTGCAAGGGACGGA GGAAGTTTCGTGCGTGCGCCCTTCCTATAGCGCCCAGTAGAACTGA CAGTACCT
883	5'	TCCTCGGATTACGCATGCTCAGTGCAATCTTCGGTTGCCTGGACTA GCGCTCCGGTTTTTCTGTGCTGAACCTCAGGGGACGCCGACACACG TACACGTC
884	5'	GATAATTCCTGAAAGAAAAGATCAATTCGATGTTACCAAATCTGG GATATCCAGAAAAATTTTCTTCTTCCTAGGAGAAAAACTATCAA ATGTCAGG
885	5'	TCTCTCACGGCAAACCTGTTGCAGACTGTAGAGACGCTATGCCAAGA ATCTTTTACTTAAAAGCAGGAATAGATTCAATAGGCAACTTCACTG CACATGTA
886	5'	CAACCTCCCCTTCTCAAGGAGCAGGTGGATTGGTCCCGAGCTAGCT GGTGGGCGGAGGTGACGTTTTTATAAGTTGCTCAAGAGACGGTAAC AACCGACG
887	5'	GTGGAACCTCCACTGAATTACTCTTTTCGCATGTAAGATCACTGAA CCGTGATAATCATTGATCCTATTTGTAGAACTGTATGAAACAGTTC CCTAAGGA

888	5'	TCGCTCAACAGGCGGCCAGGGTGCGAGCAGTGAAGCTGCGGCACGC CGGAGCGTTTAAATGGCCATCAAATTGGCCTCTCTAGGAGGTAGCTG CAGCCGGA
895	5'	AAAGGCACCTTCTCGCTGGC
896	3'	ACTTGTATGTTGTTTTT
897	5'	TCTCGCTGGC
900	5'	AGCGCTCCGGTTTTTCTGTGCTGAACCTCAGGGGACGCCGACACAC GTACACGTC

Adeno-associated virus (AAV) Vectors

[00114] In certain embodiments, an expression vector is an adeno-associated virus (AAV) vector. AAV is a small, nonenveloped icosahedral virus of the genus Dependoparvovirus and family Parvovirus. AAV has a single-stranded linear DNA genome of approximately 4.7 kb. AAV is capable of infecting both dividing and quiescent cells of several tissue types, with different AAV serotypes exhibiting different tissue tropism.

[00115] AAV includes numerous serologically distinguishable types including serotypes AAV-1 to AAV-12, as well as more than 100 serotypes from nonhuman primates (See, e.g., Srivastava (2008) J. CELL BIOCHEM., 105(1): 17–24, and Gao *et al.* (2004) J. VIROL., 78(12), 6381–6388). The serotype of the AAV vector used in the present invention can be selected by a skilled person in the art based on the efficiency of delivery, tissue tropism, and immunogenicity. For example, AAV-1, AAV-2, AAV-4, AAV-5, AAV-8, and AAV-9 can be used for delivery to the central nervous system; AAV-1, AAV-8, and AAV-9 can be used for delivery to the heart; AAV-2 can be used for delivery to the kidney; AAV-7, AAV-8, and AAV-9 can be used for delivery to the liver; AAV-4, AAV-5, AAV-6, AAV-9 can be used for delivery to the lung, AAV-8 can be used for delivery to the pancreas, AAV-2, AAV-5, and AAV-8 can be used for delivery to the photoreceptor cells; AAV-1, AAV-2, AAV-4, AAV-5, and AAV-8 can be used for delivery to the retinal pigment epithelium; AAV-1, AAV-6, AAV-7, AAV-8, and AAV-9 can be used for delivery to the skeletal muscle. In certain embodiments, the AAV capsid protein comprises a sequence as disclosed in U.S. Patent No. 7,198,951, such as, but not limited to, AAV-9 (SEQ ID NOs: 1-3 of U.S. Patent No. 7,198,951), AAV-2 (SEQ ID NO: 4 of U.S. Patent No. 7,198,951), AAV-1 (SEQ ID NO: 5 of U.S. Patent No. 7,198,951), AAV-3 (SEQ ID NO: 6 of U.S. Patent No. 7,198,951), and AAV-8 (SEQ ID NO: 7 of U.S. Patent No. 7,198,951). AAV serotypes identified from rhesus monkeys, e.g., rh.8, rh.10, rh.39, rh.43, and rh.74, are also

contemplated in the instant invention. Besides the natural AAV serotypes, modified AAV capsids have been developed for improving efficiency of delivery, tissue tropism, and immunogenicity. Exemplary natural and modified AAV capsids are disclosed in U.S. Patent Nos. 7,906,111, 9,493,788, and 7,198,951, and PCT Publication No. WO2017189964A2.

5 [00116] The wild-type AAV genome contains two 145 nucleotide inverted terminal repeats (ITRs), which contain signal sequences directing AAV replication, genome encapsidation and integration. In addition to the ITRs, three AAV promoters, p5, p19, and p40, drive expression of two open reading frames encoding rep and cap genes. Two rep promoters, coupled with differential splicing of the single AAV intron, result in the production of four rep
10 proteins (Rep 78, Rep 68, Rep 52, and Rep 40) from the rep gene. Rep proteins are responsible for genomic replication. The Cap gene is expressed from the p40 promoter, and encodes three capsid proteins (VP1, VP2, and VP3) which are splice variants of the cap gene. These proteins form the capsid of the AAV particle.

[00117] Because the *cis*-acting signals for replication, encapsidation, and integration are
15 contained within the ITRs, some or all of the 4.3 kb internal genome may be replaced with foreign DNA, for example, an expression cassette for an exogenous gene of interest. Accordingly, in certain embodiments, the AAV vector comprises a genome comprising an expression cassette for an exogenous gene flanked by a 5' ITR and a 3' ITR. The ITRs may be derived from the same serotype as the capsid or a derivative thereof. Alternatively, the ITRs
20 may be of a different serotype from the capsid, thereby generating a pseudotyped AAV. In certain embodiments, the ITRs are derived from AAV-2. In certain embodiments, the ITRs are derived from AAV-5. At least one of the ITRs may be modified to mutate or delete the terminal resolution site, thereby allowing production of a self-complementary AAV vector.

[00118] The rep and cap proteins can be provided in *trans*, for example, on a plasmid, to
25 produce an AAV vector. A host cell line permissive of AAV replication must express the rep and cap genes, the ITR-flanked expression cassette, and helper functions provided by a helper virus, for example adenoviral genes E1a, E1b55K, E2a, E4orf6, and VA (Weitzman *et al.*, Adeno-associated virus biology. Adeno-Associated Virus: Methods and Protocols, pp. 1–23, 2011). Methods for generating and purifying AAV vectors have been described in detail (See
30 *e.g.*, Mueller *et al.*, (2012) CURRENT PROTOCOLS IN MICROBIOLOGY, 14D.1.1-14D.1.21, Production and Discovery of Novel Recombinant Adeno-Associated Viral Vectors). Numerous cell types are suitable for producing AAV vectors, including HEK293 cells, COS cells, HeLa cells, BHK cells, Vero cells, as well as insect cells (See *e.g.* U.S. Patent Nos. 6,156,303,

5,387,484, 5,741,683, 5,691,176, 5,688,676, and 8,163,543, U.S. Patent Publication No. 20020081721, and PCT Publication Nos. WO00/47757, WO00/24916, and WO96/17947). AAV vectors are typically produced in these cell types by one plasmid containing the ITR-flanked expression cassette, and one or more additional plasmids providing the additional AAV and helper virus genes.

[00119] AAV of any serotype may be used in the present invention. Similarly, it is contemplated that any adenoviral type may be used, and a person of skill in the art will be able to identify AAV and adenoviral types suitable for the production of their desired recombinant AAV vector (rAAV). AAV particles may be purified, for example by affinity chromatography, iodixonal gradient, or CsCl gradient.

[00120] AAV vectors may have single-stranded genomes that are 4.7 kb in size, or are larger or smaller than 4.7 kb, including oversized genomes that are as large as 5.2 kb, or as small as 3.0 kb. Thus, where the exogenous gene of interest to be expressed from the AAV vector is small, the AAV genome may comprise a stuffer sequence. Further, vector genomes may be substantially self-complementary thereby allowing for rapid expression in the cell. In certain embodiments, the genome of a self-complementary AAV vector comprises from 5' to 3': a 5' ITR; a first nucleic acid sequence comprising a promoter and/or enhancer operably linked to a coding sequence of a gene of interest; a modified ITR that does not have a functional terminal resolution site; a second nucleic acid sequence complementary or substantially complementary to the first nucleic acid sequence; and a 3' ITR. AAV vectors containing genomes of all types are suitable for use in the method of the present invention.

[00121] Non-limiting examples of AAV vectors include pAAV-MCS (Agilent Technologies), pAAVK-EF1 α -MCS (System Bio Catalog # AAV502A-1), pAAVK-EF1 α -MCS1-CMV-MCS2 (System Bio Catalog # AAV503A-1), pAAV-ZsGreen1 (Clontech Catalog #6231), pAAV-MCS2 (Addgene Plasmid #46954), AAV-Stuffer (Addgene Plasmid #106248), pAAVscCBPIGpluc (Addgene Plasmid #35645), AAVS1_Puro_PGK1_3xFLAG_Twin_Strep (Addgene Plasmid #68375), pAAV-RAM-d2TTA::TRE-MCS-WPRE-pA (Addgene Plasmid #63931), pAAV-UbC (Addgene Plasmid #62806), pAAVS1-P-MCS (Addgene Plasmid #80488), pAAV-Gateway (Addgene Plasmid #32671), pAAV-Puro_siKD (Addgene Plasmid #86695), pAAVS1-Nst-MCS (Addgene Plasmid #80487), pAAVS1-Nst-CAG-DEST (Addgene Plasmid #80489), pAAVS1-P-CAG-DEST (Addgene Plasmid #80490), pAAVf-EnhCB-lacZnls (Addgene Plasmid #35642), and pAAVS1-shRNA (Addgene Plasmid #82697). These vectors can be modified to be suitable for therapeutic use. For example, an exogenous gene of interest

can be inserted in a multiple cloning site, and a selection marker (*e.g.*, puro or a gene encoding a fluorescent protein) can be deleted or replaced with another (same or different) exogenous gene of interest. Further examples of AAV vectors are disclosed in U.S. Patent Nos. 5,871,982, 6,270,996, 7,238,526, 6,943,019, 6,953,690, 9,150,882, and 8,298,818, U.S. Patent Publication
 5 No. 2009/0087413, and PCT Publication Nos. WO2017075335A1, WO2017075338A2, and WO2017201258A1.

[00122] In certain embodiments, the expression vector is an AAV vector capable of targeting the nervous system, *e.g.*, the central nervous system, in a subject, *e.g.*, a human subject. Exemplary AAV vectors that can target the nervous system include the AAV9 variants AAV-
 10 PHP.B (See, *e.g.*, Deverman *et al.* (2016) NAT. BIOTECHNOL. 34(2):204–209), AAV-AS (See, *e.g.*, Choudhury *et al.* (2016) MOL. THER. 24:726–35), and AAV-PHP.eB (See, *e.g.*, Chan *et al.* (2017) NAT. NEUROSCI. 20:1172–79). Additional exemplary AAV-based strategies for targeting the nervous system are described in Bedrook *et al.* (2018) ANNU REV NEUROSCI. 41:323-348. In certain embodiments, the AAV vector is an AAV-PHP.eB vector.

15 Lentivirus Vectors

[00123] In certain embodiments, the viral vector can be a retroviral vector. Examples of retroviral vectors include moloney murine leukemia virus vectors, spleen necrosis virus vectors, and vectors derived from retroviruses such as rous sarcoma virus, harvey sarcoma virus, avian leukosis virus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary
 20 tumor virus. Retroviral vectors are useful as agents to mediate retroviral-mediated gene transfer into eukaryotic cells.

[00124] In certain embodiments, the retroviral vector is a lentiviral vector. Exemplary lentiviral vectors include vectors derived from human immunodeficiency virus-1 (HIV-1), human immunodeficiency virus-2 (HIV-2), simian immunodeficiency virus (SIV), feline
 25 immunodeficiency virus (FIV), bovine immunodeficiency virus (BIV), Jembrana Disease Virus (JDV), equine infectious anemia virus (EIAV), and caprine arthritis encephalitis virus (CAEV).

[00125] Retroviral vectors typically are constructed such that the majority of sequences coding for the structural genes of the virus are deleted and replaced by the gene(s) of interest. Often, the structural genes (*i.e.*, gag, pol, and env), are removed from the retroviral backbone
 30 using genetic engineering techniques known in the art. Accordingly, a minimum retroviral vector comprises from 5' to 3': a 5' long terminal repeat (LTR), a packaging signal, an optional exogenous promoter and/or enhancer, an exogenous gene of interest, and a 3' LTR. If no exogenous promoter is provided, gene expression is driven by the 5' LTR, which is a weak

promoter and requires the presence of Tat to activate expression. The structural genes can be provided in separate vectors for manufacture of the lentivirus, rendering the produced virions replication-defective. Specifically, with respect to lentivirus, the packaging system may comprise a single packaging vector encoding the Gag, Pol, Rev, and Tat genes, and a third,
5 separate vector encoding the envelope protein Env (usually VSV-G due to its wide infectivity). To improve the safety of the packaging system, the packaging vector can be split, expressing Rev from one vector, Gag and Pol from another vector. Tat can also be eliminated from the packaging system by using a retroviral vector comprising a chimeric 5' LTR, wherein the U3 region of the 5' LTR is replaced with a heterologous regulatory element.

10 [00126] The genes can be incorporated into the proviral backbone in several general ways. The most straightforward constructions are ones in which the structural genes of the retrovirus are replaced by a single gene that is transcribed under the control of the viral regulatory sequences within the LTR. Retroviral vectors have also been constructed which can introduce more than one gene into target cells. Usually, in such vectors one gene is under the regulatory
15 control of the viral LTR, while the second gene is expressed either off a spliced message or is under the regulation of its own, internal promoter.

[00127] Accordingly, the new gene(s) are flanked by 5' and 3' LTRs, which serve to promote transcription and polyadenylation of the virion RNAs, respectively. The term "long terminal repeat" or "LTR" refers to domains of base pairs located at the ends of retroviral DNAs
20 which, in their natural sequence context, are direct repeats and contain U3, R and U5 regions. LTRs generally provide functions fundamental to the expression of retroviral genes (*e.g.*, promotion, initiation and polyadenylation of gene transcripts) and to viral replication. The LTR contains numerous regulatory signals including transcriptional control elements, polyadenylation signals, and sequences needed for replication and integration of the viral genome. The U3
25 region contains the enhancer and promoter elements. The U5 region is the sequence between the primer binding site and the R region and contains the polyadenylation sequence. The R (repeat) region is flanked by the U3 and U5 regions. In certain embodiments, the R region comprises a trans-activation response (TAR) genetic element, which interacts with the trans-activator (tat) genetic element to enhance viral replication. This element is not required in embodiments
30 wherein the U3 region of the 5' LTR is replaced by a heterologous promoter.

[00128] In certain embodiments, the retroviral vector comprises a modified 5' LTR and/or 3' LTR. Modifications of the 3' LTR are often made to improve the safety of lentiviral or retroviral systems by rendering viruses replication-defective. In specific embodiments, the

retroviral vector is a self-inactivating (SIN) vector. As used herein, a SIN retroviral vector refers to a replication-defective retroviral vector in which the 3' LTR U3 region has been modified (*e.g.*, by deletion or substitution) to prevent viral transcription beyond the first round of viral replication. This is because the 3' LTR U3 region is used as a template for the 5' LTR U3 region during viral replication and, thus, the viral transcript cannot be made without the U3 enhancer-promoter. In a further embodiment, the 3' LTR is modified such that the U5 region is replaced, for example, with an ideal polyadenylation sequence. It should be noted that modifications to the LTRs such as modifications to the 3' LTR, the 5' LTR, or both 3' and 5' LTRs, are also contemplated to be useful in the practice of the invention.

10 [00129] In certain embodiments, the U3 region of the 5' LTR is replaced with a heterologous promoter to drive transcription of the viral genome during production of viral particles. Examples of heterologous promoters which can be used include, for example, viral simian virus 40 (SV40) (*e.g.*, early or late), cytomegalovirus (CMV) (*e.g.*, immediate early), Moloney murine leukemia virus (MoMLV), Rous sarcoma virus (RSV), and herpes simplex virus (HSV) (thymidine kinase) promoters. Typical promoters are able to drive high levels of transcription in a Tat-independent manner. This replacement reduces the possibility of recombination to generate replication-competent virus, because there is no complete U3 sequence in the virus production system.

[00130] Adjacent the 5' LTR are sequences necessary for reverse transcription of the genome (the tRNA primer binding site) and for efficient packaging of viral RNA into particles (the Psi site). As used herein, the term "packaging signal" or "packaging sequence" refers to sequences located within the retroviral genome which are required for encapsidation of retroviral RNA strands during viral particle formation (*see e.g.*, Clever *et al.*, 1995 J. VIROLOGY, 69(4):2101-09). The packaging signal may be a minimal packaging signal (also referred to as the psi [Ψ] sequence) needed for encapsidation of the viral genome.

[00131] In certain embodiments, the retroviral vector (*e.g.*, lentiviral vector) further comprises a FLAP. As used herein, the term "FLAP" refers to a nucleic acid whose sequence includes the central polypurine tract and central termination sequences (cPPT and CTS) of a retrovirus, *e.g.*, HIV-1 or HIV-2. Suitable FLAP elements are described in U.S. Patent No. 6,682,907 and in Zennou *et al.* (2000) CELL, 101:173. During reverse transcription, central initiation of the plus-strand DNA at the cPPT and central termination at the CTS lead to the formation of a three-stranded DNA structure: a central DNA flap. While not wishing to be bound by any theory, the DNA flap may act as a cis-active determinant of lentiviral genome

nuclear import and/or may increase the titer of the virus. In particular embodiments, the retroviral vector backbones comprise one or more FLAP elements upstream or downstream of the heterologous genes of interest in the vectors. For example, in particular embodiments, a transfer plasmid includes a FLAP element. In one embodiment, a vector of the invention
5 comprises a FLAP element isolated from HIV-1.

[00132] In certain embodiments, the retroviral vector (*e.g.*, lentiviral vector) further comprises an export element. In one embodiment, retroviral vectors comprise one or more export elements. The term “export element” refers to a cis-acting post-transcriptional regulatory element which regulates the transport of an RNA transcript from the nucleus to the cytoplasm of
10 a cell. Examples of RNA export elements include, but are not limited to, the human immunodeficiency virus (HIV) RRE (*see e.g.*, Cullen *et al.*, (1991) J. VIROL. 65: 1053; and Cullen *et al.*, (1991) CELL 58: 423) and the hepatitis B virus post-transcriptional regulatory element (HPRE). Generally, the RNA export element is placed within the 3' UTR of a gene, and can be inserted as one or multiple copies.

15 [00133] In certain embodiments, the retroviral vector (*e.g.*, lentiviral vector) further comprises a posttranscriptional regulatory element. A variety of posttranscriptional regulatory elements can increase expression of a heterologous nucleic acid, *e.g.*, woodchuck hepatitis virus posttranscriptional regulatory element (WPRE; *see* Zufferey *et al.*, (1999) J. VIROL., 73:2886); the posttranscriptional regulatory element present in hepatitis B virus (HPRE) (Huang *et al.*,
20 MOL. CELL. BIOL., 5:3864); and the like (Liu *et al.*, (1995), GENES DEV., 9:1766). The posttranscriptional regulatory element is generally positioned at the 3' end the heterologous nucleic acid sequence. This configuration results in synthesis of an mRNA transcript whose 5' portion comprises the heterologous nucleic acid coding sequences and whose 3' portion comprises the posttranscriptional regulatory element sequence. In certain embodiments, vectors
25 of the invention lack or do not comprise a posttranscriptional regulatory element such as a WPRE or HPRE, because in some instances these elements increase the risk of cellular transformation and/or do not substantially or significantly increase the amount of mRNA transcript or increase mRNA stability. Therefore, in certain embodiments, vectors of the invention lack or do not comprise a WPRE or HPRE as an added safety measure.

30 [00134] Elements directing the efficient termination and polyadenylation of the heterologous nucleic acid transcripts increase heterologous gene expression. Transcription termination signals are generally found downstream of the polyadenylation signal. Accordingly, in certain embodiments, the retroviral vector (*e.g.*, lentiviral vector) further comprises a

polyadenylation signal. The term “polyadenylation signal” or “polyadenylation sequence” as used herein denotes a DNA sequence which directs both the termination and polyadenylation of the nascent RNA transcript by RNA polymerase H. Efficient polyadenylation of the recombinant transcript is desirable as transcripts lacking a polyadenylation signal are unstable and are rapidly degraded. Illustrative examples of polyadenylation signals that can be used in a vector of the invention, includes an ideal polyadenylation sequence (*e.g.*, AATAAA, ATTAAA AGTAAA), a bovine growth hormone polyadenylation sequence (BGHpA), a rabbit β -globin polyadenylation sequence (r β gpA), or another suitable heterologous or endogenous polyadenylation sequence known in the art.

- 10 [00135] In certain embodiments, a retroviral vector further comprises an insulator element. Insulator elements may contribute to protecting retrovirus-expressed sequences, *e.g.*, therapeutic genes, from integration site effects, which may be mediated by cis-acting elements present in genomic DNA and lead to deregulated expression of transferred sequences (*i.e.*, position effect; *see, e.g.*, Burgess-Beusse *et al.*, (2002) PROC. NATL. ACAD. SCI., USA, 99:16433; and Zhan *et al.*, 2001, HUM. GENET., 109:471). In certain embodiments, the retroviral vector
15 comprises an insulator element in one or both LTRs or elsewhere in the region of the vector that integrates into the cellular genome. Suitable insulators for use in the invention include, but are not limited to, the chicken β -globin insulator (*see* Chung *et al.*, (1993). CELL 74:505; Chung *et al.*, (1997) PROC. NATL. ACAD. SCI., USA 94:575; and Bell *et al.*, 1999. CELL 98:387).
20 Examples of insulator elements include, but are not limited to, an insulator from a β -globin locus, such as chicken HS4.

- [00136] Non-limiting examples of lentiviral vectors include pLVX-EF1alpha-AcGFP1-C1 (Clontech Catalog #631984), pLVX-EF1alpha-IRES-mCherry (Clontech Catalog #631987), pLVX-Puro (Clontech Catalog #632159), pLVX-IRES-Puro (Clontech Catalog #632186),
25 pLenti6/V5-DESTTM (Thermo Fisher), pLenti6.2/V5-DESTTM (Thermo Fisher), pLKO.1 (Plasmid #10878 at Addgene), pLKO.3G (Plasmid #14748 at Addgene), pSico (Plasmid #11578 at Addgene), pLJM1-EGFP (Plasmid #19319 at Addgene), FUGW (Plasmid #14883 at Addgene), pLVTHM (Plasmid #12247 at Addgene), pLVUT-tTR-KRAB (Plasmid #11651 at Addgene), pLL3.7 (Plasmid #11795 at Addgene), pLB (Plasmid #11619 at Addgene), pWPXL
30 (Plasmid #12257 at Addgene), pWPI (Plasmid #12254 at Addgene), EF.CMV.RFP (Plasmid #17619 at Addgene), pLenti CMV Puro DEST (Plasmid #17452 at Addgene), pLenti-puro (Plasmid #39481 at Addgene), pULTRA (Plasmid #24129 at Addgene), pLX301 (Plasmid #25895 at Addgene), pHIV-EGFP (Plasmid #21373 at Addgene), pLV-mCherry (Plasmid #36084 at Addgene), pLionII (Plasmid #1730 at Addgene), pInducer10-mir-RUP-PheS (Plasmid

#44011 at Addgene). These vectors can be modified to be suitable for therapeutic use. For example, a selection marker (*e.g.*, puro, EGFP, or mCherry) can be deleted or replaced with a second exogenous gene of interest. Further examples of lentiviral vectors are disclosed in U.S. Patent Nos. 7,629,153, 7,198,950, 8,329,462, 6,863,884, 6,682,907, 7,745,179, 7,250,299, 5,994,136, 6,287,814, 6,013,516, 6,797,512, 6,544,771, 5,834,256, 6,958,226, 6,207,455, 6,531,123, and 6,352,694, and PCT Publication No. WO2017/091786.

Adenoviral Vectors

[00137] In certain embodiments, the viral vector can be an adenoviral vector. Adenoviruses are medium-sized (90-100 nm), non-enveloped (naked), icosahedral viruses composed of a nucleocapsid and a double-stranded linear DNA genome. The term "adenovirus" refers to any virus in the genus Adenoviridae including, but not limited to, human, bovine, ovine, equine, canine, porcine, murine, and simian adenovirus subgenera. Typically, an adenoviral vector is generated by introducing one or more mutations (*e.g.*, a deletion, insertion, or substitution) into the adenoviral genome of the adenovirus so as to accommodate the insertion of a non-native nucleic acid sequence, for example, for gene transfer, into the adenovirus.

[00138] A human adenovirus can be used as the source of the adenoviral genome for the adenoviral vector. For instance, an adenovirus can be of subgroup A (*e.g.*, serotypes 12, 18, and 31), subgroup B (*e.g.*, serotypes 3, 7, 11, 14, 16, 21, 34, 35, and 50), subgroup C (*e.g.*, serotypes 1, 2, 5, and 6), subgroup D (*e.g.*, serotypes 8, 9, 10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, and 42-48), subgroup E (*e.g.*, serotype 4), subgroup F (*e.g.*, serotypes 40 and 41), an unclassified serogroup (*e.g.*, serotypes 49 and 51), or any other adenoviral serogroup or serotype. Adenoviral serotypes 1 through 51 are available from the American Type Culture Collection (ATCC, Manassas, Virginia). Non-group C adenoviral vectors, methods of producing non-group C adenoviral vectors, and methods of using non-group C adenoviral vectors are disclosed in, for example, U.S. Patent Nos. 5,801,030, 5,837,511, and 5,849,561, and PCT Publication Nos. WO1997/012986 and WO1998/053087.

[00139] Non-human adenovirus (*e.g.*, ape, simian, avian, canine, ovine, or bovine adenoviruses) can be used to generate the adenoviral vector (*i.e.*, as a source of the adenoviral genome for the adenoviral vector). For example, the adenoviral vector can be based on a simian adenovirus, including both new world and old world monkeys (see, *e.g.*, Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses (2005)). A phylogeny analysis of adenoviruses that infect primates is disclosed in, *e.g.*, Roy *et al.* (2009) PLOS PATHOG. 5(7):e1000503. A gorilla adenovirus can be used as the source of the adenoviral

genome for the adenoviral vector. Gorilla adenoviruses and adenoviral vectors are described in, *e.g.*, PCT Publication Nos. WO2013/052799, WO2013/052811, and WO2013/052832. The adenoviral vector can also comprise a combination of subtypes and thereby be a "chimeric" adenoviral vector.

5 **[00140]** The adenoviral vector can be replication-competent, conditionally replication-competent, or replication-deficient. A replication-competent adenoviral vector can replicate in typical host cells, *i.e.*, cells typically capable of being infected by an adenovirus. A conditionally-replicating adenoviral vector is an adenoviral vector that has been engineered to replicate under pre-determined conditions. For example, replication-essential gene functions,
10 *e.g.*, gene functions encoded by the adenoviral early regions, can be operably linked to an inducible, repressible, or tissue-specific transcription control sequence, *e.g.*, a promoter. Conditionally-replicating adenoviral vectors are further described in U.S. Patent No. 5,998,205. A replication-deficient adenoviral vector is an adenoviral vector that requires complementation of one or more gene functions or regions of the adenoviral genome that are required for
15 replication, as a result of, for example, a deficiency in one or more replication-essential gene function or regions, such that the adenoviral vector does not replicate in typical host cells, especially those in a human to be infected by the adenoviral vector.

[00141] Preferably, the adenoviral vector is replication-deficient, such that the replication-deficient adenoviral vector requires complementation of at least one replication-essential gene
20 function of one or more regions of the adenoviral genome for propagation (*e.g.*, to form adenoviral vector particles). The adenoviral vector can be deficient in one or more replication-essential gene functions of only the early regions (*i.e.*, E1-E4 regions) of the adenoviral genome, only the late regions (*i.e.*, L1-L5 regions) of the adenoviral genome, both the early and late regions of the adenoviral genome, or all adenoviral genes (*i.e.*, a high capacity adenovector (HC-
25 Ad)). See, *e.g.*, Morsy *et al.* (1998) PROC. NATL. ACAD. SCI. USA 95: 965-976, Chen *et al.* (1997) PROC. NATL. ACAD. SCI. USA 94: 1645-1650, and Kochanek *et al.* (1999) HUM. GENE THER. 10(15):2451-9. Examples of replication-deficient adenoviral vectors are disclosed in U.S. Patent Nos. 5,837,511, 5,851,806, 5,994,106, 6,127,175, 6,482,616, and 7,195,896, and PCT Publication Nos. WO1994/028152, WO1995/002697, WO1995/016772, WO1995/034671,
30 WO1996/022378, WO1997/012986, WO1997/021826, and WO2003/022311.

[00142] The replication-deficient adenoviral vector of the invention can be produced in complementing cell lines that provide gene functions not present in the replication-deficient adenoviral vector, but required for viral propagation, at appropriate levels in order to generate

high titers of viral vector stock. Such complementing cell lines are known and include, but are not limited to, 293 cells (described in, *e.g.*, Graham *et al.* (1977) J. GEN. VIROL. 36: 59-72), PER.C6 cells (described in, *e.g.*, PCT Publication No. WO1997/000326, and U.S. Patent Nos. 5,994,128 and 6,033,908), and 293-ORF6 cells (described in, *e.g.*, PCT Publication No. WO1995/034671 and Brough *et al.* (1997) J. VIROL. 71: 9206-9213). Other suitable complementing cell lines to produce the replication-deficient adenoviral vector of the invention include complementing cells that have been generated to propagate adenoviral vectors encoding transgenes whose expression inhibits viral growth in host cells (see, *e.g.*, U.S. Patent Publication No. 2008/0233650). Additional suitable complementing cells are described in, for example, U.S. Patent Nos. 6,677,156 and 6,682,929, and PCT Publication No. WO2003/020879. Formulations for adenoviral vector-containing compositions are further described in, for example, U.S. Patent Nos. 6,225,289, and 6,514,943, and PCT Publication No. WO2000/034444.

[00143] Additional exemplary adenoviral vectors, and/or methods for making or propagating adenoviral vectors are described in U.S. Patent Nos. 5,559,099, 5,837,511, 5,846,782, 5,851,806, 5,994,106, 5,994,128, 5,965,541, 5,981,225, 6,040,174, 6,020,191, 6,083,716, 6,113,913, 6,303,362, 7,067,310, and 9,073,980.

[00144] Commercially available adenoviral vector systems include the ViraPower™ Adenoviral Expression System available from Thermo Fisher Scientific, the AdEasy™ adenoviral vector system available from Agilent Technologies, and the Adeno-X™ Expression System 3 available from Takara Bio USA, Inc.

Viral Vector Production

[00145] Methods for producing viral vectors are known in the art. Typically, a virus of interest is produced in a suitable host cell line using conventional techniques including culturing a transfected or infected host cell under suitable conditions so as to allow the production of infectious viral particles. Nucleic acids encoding viral genes and/or tRNAs can be incorporated into plasmids and introduced into host cells through conventional transfection or transformation techniques. Exemplary suitable host cells for production of disclosed viruses include human cell lines such as HeLa, Hela-S3, HEK293, 911, A549, HER96, or PER-C6 cells. Specific production and purification conditions will vary depending upon the virus and the production system employed.

[00146] In certain embodiments, producer cells may be directly administered to a subject, however, in other embodiments, following production, infectious viral particles are recovered

from the culture and optionally purified. Typical purification steps may include plaque purification, centrifugation, *e.g.*, cesium chloride gradient centrifugation, clarification, enzymatic treatment, *e.g.*, benzonase or protease treatment, chromatographic steps, *e.g.*, ion exchange chromatography or filtration steps.

5 **IV. Pharmaceutical Compositions**

[00147] For therapeutic use, a tRNA and/or expression vector preferably is combined with a pharmaceutically acceptable carrier. The term “pharmaceutically acceptable” as used herein refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings
10 and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00148] The term “pharmaceutically acceptable carrier” as used herein refers to buffers, carriers, and excipients suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication,
15 commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable carriers include any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (*e.g.*, such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see, *e.g.*, Martin, Remington’s Pharmaceutical Sciences, 15th Ed.,
20 Mack Publ. Co., Easton, PA [1975]. Pharmaceutically acceptable carriers include buffers, solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is known in the art.

[00149] In certain embodiments, a pharmaceutical composition may contain formulation
25 materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. In such embodiments, suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite);
30 buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides; and

other carbohydrates (such as glucose, mannose or dextrans); proteins (such as serum albumin, gelatin or immunoglobulins); coloring, flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (such as sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants (See *Remington's Pharmaceutical Sciences*, 18th ed. (Mack Publishing Company, 1990).

[00150] In certain embodiments, a pharmaceutical composition may contain nanoparticles, *e.g.*, polymeric nanoparticles, liposomes, or micelles (See Anselmo *et al.* (2016) BIOENG. TRANSL. MED. 1: 10-29). In certain embodiments, the composition does not comprise (or is substantially free of, for example, the composition comprises less than 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of) a nanoparticle or an aminolipid delivery compound, *e.g.*, as described in U.S. Patent Publication No. 2017/0354672. In certain embodiments, the tRNA or expression vector introduced into the cell or administered to the subject is not conjugated to or associated with another moiety, *e.g.*, a carrier particle, *e.g.*, an aminolipid particle. As used herein, the term "conjugated," when used with respect to two or more moieties, means that the moieties are physically associated or connected with one another, either directly or via one or more additional moieties that serves as a linking agent, to form a structure that is sufficiently stable so that the moieties remain physically associated under the conditions in which structure is used, *e.g.*, physiological conditions. Typically the moieties are attached either by one or more covalent bonds or by a mechanism that involves specific binding. Alternately, a sufficient number of weaker interactions can provide sufficient stability for moieties to remain physically associated.

[00151] In certain embodiments, a pharmaceutical composition may contain a sustained- or controlled-delivery formulation. Techniques for formulating sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. Sustained-release preparations may include, *e.g.*, porous polymeric microparticles or semipermeable polymer matrices in the form of shaped articles, *e.g.*, films, or microcapsules. Sustained release matrices may include polyesters,

hydrogels, polylactides, copolymers of L-glutamic acid and gamma ethyl-L-glutamate, poly (2-hydroxyethyl-inethacrylate), ethylene vinyl acetate, or poly-D(-)-3-hydroxybutyric acid.

Sustained release compositions may also include liposomes that can be prepared by any of several methods known in the art.

- 5 [00152] Pharmaceutical compositions containing a tRNA and/or expression vector disclosed herein can be presented in a dosage unit form and can be prepared by any suitable method. A pharmaceutical composition should be formulated to be compatible with its intended route of administration. Examples of routes of administration are intravenous (IV), intradermal, inhalation, transdermal, topical, transmucosal, intrathecal and rectal administration. In certain
10 embodiments, a tRNA and/or expression vector is administered intrathecally. In certain embodiments, a tRNA and/or expression vector is administered by injection. Useful formulations can be prepared by methods known in the pharmaceutical art. For example, see *Remington's Pharmaceutical Sciences*, 18th ed. (Mack Publishing Company, 1990). Formulation components suitable for parenteral administration include a sterile diluent such as
15 water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as EDTA; buffers such as acetates, citrates or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose.
- 20 [00153] For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). The carrier should be stable under the conditions of manufacture and storage, and should be preserved against microorganisms. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and
25 liquid polyethylene glycol), and suitable mixtures thereof.

- [00154] In general, any method of delivering a nucleic acid molecule can be adapted for use with a tRNA (see *e.g.*, Akhtar *et al.* (1992) *TRENDS CELL. BIOL.* 2(5):139-144 and PCT Publication No. WO94/02595). The tRNA can be modified or alternatively delivered using a drug delivery system to prevent the rapid degradation of the tRNA by endo- and exo-nucleases
30 *in vivo*. tRNA molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. tRNA molecules can also be conjugated to or otherwise associated with an aptamer. A tRNA can also be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic

delivery system. Positively charged cationic delivery systems facilitate binding of a tRNA molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of a tRNA by the cell. Cationic lipids, dendrimers, or polymers can either be bound to the RNA, *e.g.*, tRNA, or induced to form a vesicle or micelle (see *e.g.*, Kim *et al.* (2008) JOURNAL OF CONTROLLED RELEASE 129(2):107-116) that encases the RNA. Methods for making and administering cationic-RNA complexes are well within the abilities of one skilled in the art (see, *e.g.*, Sorensen *et al.* (2003) J. MOL. BIOL 327:761-766; Verma *et al.* (2003) CLIN. CANCER RES. 9:1291-1300; Arnold *et al.* (2007) J. HYPERTENS. 25:197-205). Some non-limiting examples of drug delivery systems useful for systemic delivery of RNAs, *e.g.*, tRNAs include DOTAP (Sorensen *et al.* (2003) *supra*; Verma *et al.* (2003), *supra*), Oligofectamine, solid nucleic acid lipid particles (Zimmermann *et al.* (2006) NATURE 441:111-114), cardiolipin (Chien *et al.* (2005) CANCER GENE THER. 12:321-328; Pal *et al.* (2005) INT J. ONCOL. 26:1087-1091), polyethyleneimine (Bonnet *et al.* (2008) PHARM. RES. 25(12):2972-82; Aigner (2006) J. BIOMED. BIOTECHNOL. 71659), Arg-Gly-Asp (RGD) peptides (Liu (2006) MOL. PHARM. 3:472-487), and polyamidoamines (Tomalia *et al.* (2007) BIOCHEM. SOC. TRANS. 35:61-67; Yoo *et al.* (1999) PHARM. RES. 16:1799-1804). In certain embodiments, a tRNA forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of RNAs and cyclodextrins can be found in U.S. Patent No. 7,427,605.

[00155] Pharmaceutical formulations preferably are sterile. Sterilization can be accomplished by any suitable method, *e.g.*, filtration through sterile filtration membranes. Where the composition is lyophilized, filter sterilization can be conducted prior to or following lyophilization and reconstitution.

[00156] The compositions described herein may be administered locally or systemically. Administration will generally be parenteral administration. In a preferred embodiment, the pharmaceutical composition is administered subcutaneously and in an even more preferred embodiment intravenously. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions.

[00157] Generally, a therapeutically effective amount of active component, for example, a tRNA and/or expression vector, is in the range of 0.1 mg/kg to 100 mg/kg, *e.g.*, 1 mg/kg to 100 mg/kg, 1 mg/kg to 10 mg/kg. In certain embodiments, a therapeutically effective amount of a viral expression vector is in the range of 10^2 to 10^{15} plaque forming units (pfus), *e.g.*, 10^2 to 10^{10} , 10^2 to 10^5 , 10^5 to 10^{15} , 10^5 to 10^{10} , or 10^{10} to 10^{15} plaque forming units. The amount administered

will depend on variables such as the type and extent of disease or indication to be treated, the overall health of the patient, the *in vivo* potency of the antibody, the pharmaceutical formulation, and the route of administration. The initial dosage can be increased beyond the upper level in order to rapidly achieve the desired blood-level or tissue-level. Alternatively, the initial dosage
 5 can be smaller than the optimum, and the daily dosage may be progressively increased during the course of treatment. Human dosage can be optimized, *e.g.*, in a conventional Phase I dose escalation study designed to run from 0.5 mg/kg to 20 mg/kg. Dosing frequency can vary, depending on factors such as route of administration, dosage amount, serum half-life, and the disease being treated. Exemplary dosing frequencies are once per day, once per week and once
 10 every two weeks. A preferred route of administration is parenteral, *e.g.*, intravenous infusion. In certain embodiments, a polypeptide and/or multimeric protein is lyophilized, and then reconstituted in buffered saline, at the time of administration.

[00158] In certain embodiments, the tRNA or expression vector is not conjugated to or associated with another moiety, *e.g.*, a carrier particle, *e.g.*, an aminolipid particle. In certain
 15 embodiments, the tRNA or expression vector is introduced into the cell or administered to subject in a dosage form lacking a nanoparticle. In certain embodiments, the tRNA or expression vector is introduced into the cell or administered to subject in a dosage form lacking an aminolipid delivery compound, *e.g.*, as described in U.S. Patent Publication No. 2017/0354672.

20 V. Therapeutic Uses

[00159] The compositions and methods disclosed herein can be used to treat a premature termination codon (PTC)-mediated disorder in a subject. As used herein, the term “PTC-mediated disorder” refers to a disorder that is mediated, enhanced, exacerbated, or otherwise facilitated by or associated with a PTC in a gene.

25 [00160] The invention provides a method of treating a PTC-mediated disorder in a subject in need thereof. The method comprises administering to the subject an effective amount of a tRNA and/or expression vector, *e.g.*, a tRNA and/or expression vector disclosed herein, either alone or in a combination with another therapeutic agent to treat the PTC-mediated disorder in the subject.

30 [00161] In certain embodiments, the premature termination codon-mediated disorder is a disorder listed in **TABLE 5** below, and/or the gene with a premature termination codon is a gene listed in the corresponding row of **TABLE 5** below.

TABLE 5

Gene	Disorder
SCN1A	Dravet Syndrome; Genetic Epilepsy with Febrile Seizures (GEFS)
KCNQ2	Benign Familial Infantile Epilepsy (BFIE); Early Infantile Epileptic Encephalopathy (EIEE)
SCN2A	Benign Familial Infantile Epilepsy (BFIE); Early Infantile Epileptic Encephalopathy (EIEE)
CDKL5	Early Infantile Epileptic Encephalopathy (EIEE); Lennox-Gastaut Syndrome; CDKL5 deficiency disorder
MECP2	Rett Syndrome; PPM-X Syndrome
STXBP1	Early Infantile Epileptic Encephalopathy (EIEE); Ohtahara Syndrome; Dravet Syndrome
SCN8A	Benign Familial Infantile Epilepsy (BFIE); Early Infantile Epileptic Encephalopathy (EIEE)
CACNA1A	Episodic Ataxia; Hemiplegic Migraine
SLC2A1	Idiopathic Generalized Epilepsy
FOXG1	FOXG1 Syndrome
PCDH19	Early Infantile Epileptic Encephalopathy (EIEE)
GRIN2B	Early Infantile Epileptic Encephalopathy (EIEE)
DEPDC5	Familial Focal Epilepsy with Variable Foci (FFEVF)
GRIN2A	Early Infantile Epileptic Encephalopathy (EIEE); Lennox-Gastaut Syndrome
CHD2	Childhood-onset epileptic encephalopathy
SCN9A	Congenital insensitivity to pain, etc
SYNGAP1	SYNGAP1-related intellectual disability
ALDH7A1	Pyridoxine-dependent epilepsy
GRIN1	Early Infantile Epileptic Encephalopathy (EIEE); Lennox-Gastaut Syndrome
TBC1D24	Early Infantile Epileptic Encephalopathy (EIEE); Familial Infantile Myoclonic Epilepsy (FIME)
SLC6A1	Myoclonic Astatic Epilepsy
DNM1	Early Infantile Epileptic Encephalopathy (EIEE)

ARX	Early Infantile Epileptic Encephalopathy (EIEE); X-linked Intellectual Disability
KCNB1	Early Infantile Epileptic Encephalopathy (EIEE)
KCNA1	Partial Epilepsy and Episodic Ataxia
GABRG2	Genetic Epilepsy with Febrile Seizures (GEFS); Early Infantile Epileptic Encephalopathy (EIEE); Febrile seizures
WWOX	Early Infantile Epileptic Encephalopathy (EIEE)
GABRB3	Early Infantile Epileptic Encephalopathy (EIEE); Lennox-Gastaut Syndrome
SZT2	Early Infantile Epileptic Encephalopathy (EIEE)
LGI1	Autosomal Dominant Partial Epilepsy with Auditory Features (ADPEAF)
PNPO	PNPO-Deficiency
SCN1B	Genetic Epilepsy with Febrile Seizures (GEFS); Early Infantile Epileptic Encephalopathy (EIEE)
UBA5	Early Infantile Epileptic Encephalopathy (EIEE)
KCTD7	Progressive Myoclonus Epilepsy
SCARB2	Action Myoclonus – Renal Failure (AMRF); Progressive Myoclonic Epilepsy
SLC13A5	Early Infantile Epileptic Encephalopathy (EIEE)
CSTB	Progressive Myoclonic Epilepsy
EPM2A	Progressive Myoclonic Epilepsy
PRRT2	Benign Familial Infantile Seizures (BFIS)
NHLRC1	Progressive Myoclonic Epilepsy
SLC25A22	Early Infantile Epileptic Encephalopathy (EIEE)
PRRT2	Benign Familial Infantile Seizures (BFIS)
ALG13	Early Infantile Epileptic Encephalopathy (EIEE)

[00162] In certain embodiments, the premature termination codon-mediated disorder is a disorder listed in **TABLE 6** below, and/or the gene with a premature termination codon is a gene listed in the corresponding row of **TABLE 6** below.

TABLE 6

Gene	Disorder
β -globin	β -thalassemia
CHM	Choroideremia
CFTR	Cystic Fibrosis
dystrophin	Duchenne Muscular Dystrophy
α -L-iduronidase	Hurler Syndrome
KIF1A	KIF1A
FBN1	Marfan Syndrome
ARSB	Maroteaux-Lamy Syndrome
SMPD1	Niemann Pick Disease
NAGLU	Sanfilippo Syndrome
DHCR7	Smith-Lemli-Opitz Syndrome
SCN5A	Brugada Syndrome
KCNH2 (hERG)	Long QT Syndrome type 2
KCNQ1	Long QT Syndrome type 1
TTN	Dilated Cardiomyopathy
MYBPC3	Familial Hypertrophic Cardiomyopathy
LMNA	Dilated Cardiomyopathy (sometimes Emery-Dreifuss Muscular Dystrophy)
PKP2	Familial Arrhythmogenic Right Ventricular Dysplasia
PLN	Familial Isolated Dilated Cardiomyopathy
TSC1/2	Tuberous Sclerosis
LDLR	Familial Hypercholesterolemia
SMN1	Spinal Muscular Atrophy

[00163] In certain embodiments, the PTC-mediated disorder is an epilepsy (*e.g.*, Dravet syndrome), wherein the method reduces seizure frequency, seizure severity, and/or cognitive impairment in the subject. For example, in certain embodiments, the method reduces seizure frequency in the subject by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% over the period of, *e.g.*, a day, a week, or a month. In certain embodiments, the method reduces seizure frequency by 50% over the period of, *e.g.*, a day, a week, or a month.

[00164] In certain embodiments, the PTC-mediated disorder is Dravet and/or the gene with a premature termination codon is SCN1A. In certain embodiments, a premature termination codon in the SCN1A gene is caused by a mutation, or a combination of mutations, selected from

c.5745C>G, c.5713G>T, c.5701C>T, c.5677C>T, c.5641C>T, c.5629C>T, c.5623C>T, c.5503A>T, c.5473G>T, c.5437G>T, c.5428C>T, c.5403G>A, c.5402G>A, c.5383G>T, c.5371G>T, c.5049T>G, c.4921G>T, c.4900C>T, c.4873C>T, c.4779del, c.4778G>A, c.4774G>T, c.4761T>G, c.4648G>T, c.4540C>T, c.4516A>T, c.4514C>A, c.4508T>G, c.4488C>G, c.4471G>T, c.4300A>T, c.4269G>A, c.4268G>A, c.4233T>A, c.4222G>T, c.4191G>A, c.4190G>A, c.4186C>T, c.4159A>T, c.4155C>A, c.3964del, c.3952C>T, c.3825G>A, c.3824G>A, c.3819G>A, c.3818G>A, c.3795T>A, c.3789T>G, c.3779G>A, c.3750C>G, c.3724G>T, c.3700C>T, c.3697C>T, c.3657dup, c.3624G>A, c.3604C>T, c.3582G>A, c.3578G>A, c.3574C>T, c.3463C>T, c.3454del, c.3424G>T, c.3422C>A, c.3406G>T, c.3328G>T, c.3273C>A, c.3262G>T, c.3073C>T, c.3060T>A, c.2844T>A, c.2749C>T, c.2695C>T, c.2645T>A, c.2560C>T, c.2551C>T, c.2546C>A, c.2462G>A, c.2298del, c.2228G>A, c.2181G>A, c.2180G>A, c.2101C>T, c.2038A>T, c.1958T>A, c.1837C>T, c.1834C>T, c.1804G>T, c.1795G>T, c.1738C>T, c.1702C>T, c.1660C>T, c.1624C>T, c.1516C>T, c.1378C>T, c.1363C>T, c.1354A>T, c.1348C>T, c.1345G>T, c.1344dup, c.1306G>T, c.1278C>A, c.1278C>G, c.1151G>A, c.1129C>T, c.1118T>A, c.942del, c.751del, c.644T>A, c.327C>G, c.249C>A, c.121A>T, c.4846_4850dup, c.4787_4788del, c.4578_4612dup, c.4211_4212del, c.4125_4130delinsATAATCATACTGATTGCCTAAACTAAT, c.3690_3693del, c.3338_3339del, c.1247_1248insGTAGA, c.825_826insGTATA, and c.278_279dup. In certain embodiments, a premature termination codon in the SCN1A gene is caused by a mutation, or a combination of mutations, selected from c.58G>T, c.575G>A, c.664C>T, c.962C>G, c.1095dupT, c.1129C>T, c.1315C>T, c.1348C>T, c.1366G>T, c.1492A>T, c.1537G>T, c.1624C>T, c.1738C>T, c.1804G>T, c.1837C>T, c.2134C>T, c.2370T>A, c.2495G>A, c.2593C>T, c.2635delC, c.2904C>A, c.3295G>T, c.3311C>A, c.3452C>G, c.3637C>T, c.3656G>A, c.3733C>T, c.3783C>A, c.3829C>T, c.3985C>T, c.4359T>G, c.4547C>A,

c.4573C>T, c.4721C>G, c.4954G>T, c.5641G>T, c.5656C>T, and c.5734C>T. In certain
embodiments, a premature termination codon in the SCN1A gene is caused by a mutation
selected from c.664C>T, c.1129C>T, c.1492A>T, c.1624C>T, c.1738C>T, c.1837C>T,
c.2134C>T, c.2593C>T, c.3637C>T, c.3733C>T, c.3985C>T, c.4573C>T, c.5656C>T, and
5 c.5734C>T. In certain embodiments, a premature termination codon in the SCN1A gene is
caused by a mutation selected from c.1738C>T and c.3985C>T.

[00165] In certain embodiments, a premature termination codon in the SCN1A gene is
caused by a mutation set forth in **TABLE 7**, or a combination of mutations set forth in **TABLE**
7.

10

TABLE 7

Mutation (coding DNA)	Mutation (Protein)	Suppressor Class
c.664C>T	Arg222Ter	Arg>TGA
c.3637C>T	Arg1213Ter	Arg>TGA
c.3733C>T	Arg1245Ter	Arg>TGA
c.2134C>T	Arg712Ter	Arg>TGA
c.1837C>T	Arg613Ter	Arg>TGA
c.4188C>A	Cys1396Ter	Cys>TGA
c.2877T>A	Cys959Ter	Cys>TGA
c.3183T>A	Cys1061Ter	Cys>TGA
c.3607C>T	Gln1203Ter	Gln>TAA
c.2782C>T	Gln928Ter	Gln>TAA
c.3829C>T	Gln1277Ter	Gln>TAA
c.2893C>T	Gln965Ter	Gln>TAA
c.3106C>T	Gln1036Ter	Gln>TAG
c.3496C>T	Gln1166Ter	Gln>TAG
c.5662C>T	Gln1888Ter	Gln>TAG
c.5461C>T	Gln1821Ter	Gln>TAG
c.3730C>T	Gln1244Ter	Gln>TAG
c.5506G>T	Glu1836Ter	Glu>TAA
c.5470G>T	Glu1824Ter	Glu>TAA
c.3757G>T	Glu1253Ter	Glu>TAA
c.3439G>T	Glu1147Ter	Glu>TAA

Mutation (coding DNA)	Mutation (Protein)	Suppressor Class
c.1345G>T	Glu449Ter	Glu>TAA
c.5404G>T	Glu1802Ter	Glu>TAG
c.1804G>T	Glu602Ter	Glu>TAG
c.5416G>T	Glu1806Ter	Glu>TAG
c.1795G>T	Glu599Ter	Glu>TAG
c.1549G>T	Glu517Ter	Glu>TAG
c.4255G>T	Gly1419Ter	Gly>TGA
c.4954G>T	Gly1652Ter	Gly>TGA
c.4807G>T	Gly1603Ter	Gly>TGA
c.487G>T	Gly163Ter	Gly>TGA
c.1843G>T	Gly615Ter	Gly>TGA
c.539T>A	Leu180Ter	Leu>TAA
c.2678T>A	Leu893Ter	Leu>TAA
c.644T>A	Leu215Ter	Leu>TAG
c.1958T>A	Leu653Ter	Leu>TAG
c.1118T>A	Leu373Ter	Leu>TAG
c.4541T>G	Leu1514Ter	Leu>TGA
c.2627T>G	Leu876Ter	Leu>TGA
c.4549A>T	Lys1517Ter	Lys>TAA
c.5536A>T	Lys1846Ter	Lys>TAA
c.121A>T	Lys41Ter	Lys>TAA
c.4192A>T	Lys1398Ter	Lys>TAA
c.1354A>T	Lys452Ter	Lys>TAA
c.2071A>T	Lys691Ter	Lys>TAG
c.3455C>A	Ser1152Ter	Ser>TAA
c.2579C>A	Ser860Ter	Ser>TAA
c.1883C>A	Ser628Ter	Ser>TAA
c.4547C>A	Ser1516Ter	Ser>TAG
c.2213G>A	Trp738Ter	Trp>TAG
c.3611G>A	Trp1204Ter	Trp>TAG
c.4811G>A	Trp1604Ter	Trp>TAG

Mutation (coding DNA)	Mutation (Protein)	Suppressor Class
c.4223G>A	Trp1408Ter	Trp>TAG
c.5435G>A	Trp1812Ter	Trp>TAG
c.3615G>A	Trp1205Ter	Trp>TGA
c.4224G>A	Trp1408Ter	Trp>TGA
c.4302G>A	Trp1434Ter	Trp>TGA
c.3858G>A	Trp1286Ter	Trp>TGA
c.5436G>A	Trp1812Ter	Trp>TGA
c.3762T>A	Tyr1254Ter	Tyr>TAA
c.3828T>A	Tyr1276Ter	Tyr>TAA
c.4266T>A	Tyr1422Ter	Tyr>TAA
c.3306C>A	Tyr1102Ter	Tyr>TAA
c.249C>A	Tyr83Ter	Tyr>TAA
c.5082T>G	Tyr1694Ter	Tyr>TAG
c.4794T>G	Tyr1598Ter	Tyr>TAG
c.4521C>G	Tyr1507Ter	Tyr>TAG
c.3822T>G	Tyr1274Ter	Tyr>TAG
c.5778C>G	Tyr1926Ter	Tyr>TAG

[00166] Additional exemplary mutations, including exemplary mutations causing a premature termination codon in a gene, *e.g.*, the SCN1A gene, can be found in ClinVar (available on the world wide web at ncbi.nlm.nih.gov/clinvar/), “A catalog of SCN1A variants” Lossin *et al.* (2009) BRAIN DEV. 2009 31(2):114-30, the SCN1A Registry (available on the world wide web at scn1a.net/scn1a-registry/), the SCN1A Mutation Database (available on the world wide web at gzneurosci.com/scn1adatabase), and the Leiden Open Variation Database (LOVD v.3.0; available on the world wide web at databases.lovd.nl/shared/genes/SCN1A). Unless indicated otherwise, any SCN1A mutations described herein are relative to SCN1a isoform 1 (NCBI reference sequence NM_001165963, SEQ ID NO: 863).

10 [00167] In another aspect, the invention provides a method of treating Dravet syndrome in a subject in need thereof wherein the subject has a SCN1A gene with a mutation set forth in a row **TABLE 7**, the method comprising administering to the subject an effective amount of a suppressor tRNA of the suppressor class indicated in the same row of **TABLE 7** as the mutation, or an expression vector comprising a nucleotide sequence encoding the tRNA. “Suppressor

Class” as used in TABLE 7 (*e.g.*, Arg>TGA) refers to the endogenous tRNA type from which the suppressor tRNA is derived (*e.g.*, an arginine tRNA) and the termination codon recognized by the suppressor tRNA (*e.g.*, TGA). Exemplary Arg>TGA suppressor tRNAs include tRNAs comprising a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 19-22, and 35.

- 5 Exemplary Gln>TAA suppressor tRNAs include tRNAs comprising a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45. Exemplary Gln>TAG suppressor tRNAs include tRNAs comprising a nucleotide sequence selected from SEQ ID NOs: 178-182, 186, and 187.

[00168] For example, in certain embodiments, the subject has a SCN1A gene with a premature termination codon selected from c.664C>T, c.3637C>T, c.3733C>T, c.2134C>T, and
 10 c.1837C>T, and the method comprises administering to the subject an effective amount of a suppressor tRNA comprising a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 19-22, and 35. In certain embodiments, the subject has a SCN1A gene with a premature termination codon selected from c.3607C>T, c.2782C>T, c.3829C>T, and c.2893C>T, and the method comprises administering to the subject an effective amount of a suppressor tRNA
 15 comprising a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45. In certain embodiments, the subject has a SCN1A gene with a premature termination codon selected from c.3106C>T, c.3496C>T, c.5662C>T, c.5461C>T, and c.3730C>T, and the method comprises administering to the subject an effective amount of a suppressor tRNA comprising a nucleotide sequence selected from SEQ ID NOs: 178-182, 186, and 187.

20 [00169] In certain embodiments, wherein the gene is a SCN1A gene, the SCN1A gene product produced with the tRNA is a functional SCN1A gene product. In certain embodiments, the functional SCN1A gene product has greater activity than the truncated SCN1A gene product, *e.g.*, greater voltage-gated sodium channel activity. In certain embodiments, the method increases voltage-gated sodium channel activity in a cell, tissue, or subject by about 10%, about
 25 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 110%, about 120%, about 130%, about 140%, about 150%, about 160%, about 170%, about 180%, about 190%, about 200%, about 250%, about 300%, about 350%, about 400%, about 450%, about 500%, about 600%, about 700%, about 800%, about 900%, or about 1000% relative to a cell, tissue, or subject without the tRNA. In certain embodiments, the
 30 method increases voltage-gated sodium channel activity in a cell, tissue, or subject by from about 20% to about 200%, about 20% to about 180%, about 20% to about 160%, about 20% to about 140%, about 20% to about 120%, about 20% to about 100%, about 20% to about 80%, about 20% to about 60%, about 20% to about 40%, about 40% to about 200%, about 40% to about 180%, about 40% to about 160%, about 40% to about 140%, about 40% to about 120%,

about 40% to about 100%, about 40% to about 80%, about 40% to about 60%, about 60% to about 200%, about 60% to about 180%, about 60% to about 160%, about 60% to about 140%, about 60% to about 120%, about 60% to about 100%, about 60% to about 80%, about 80% to about 200%, about 80% to about 180%, about 80% to about 160%, about 80% to about 140%, about 80% to about 120%, about 80% to about 100%, about 100% to about 200%, about 100% to about 180%, about 100% to about 160%, about 100% to about 140%, about 100% to about 120%, about 120% to about 200%, about 120% to about 180%, about 120% to about 160%, about 120% to about 140%, about 140% to about 200%, about 140% to about 180%, about 140% to about 160%, about 160% to about 200%, about 160% to about 180%, or about 180% to about 200% relative to a cell, tissue, or subject without the tRNA. Voltage-gated sodium channel activity may be measured by any method known in the art, for example, as described in Kalume *et al.* (2007) J. NEUROSCI. 27(41):11065-74, Yu *et al.* (2007) NAT. NEUROSCI. 9(9): 1142-9, and Han *et al.* (2012) NATURE 489(7416): 385–390.

[00170] In certain embodiments, the functional SCN1A gene product is the Nav1.1 protein. In certain embodiments, the functional SCN1A gene product comprises, consists essentially of, or consists of the amino acid sequence of any one of the following amino acid sequences, or an amino acid sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of the following amino acid sequences (each corresponding to different isoforms of SCN1A) :

MEQTVLVPPGPDSFNFFTRSLAAIERRIAEEKAKNPKPKDKDDENGPKPNSDLEAGKNLPFI
YGDIPPEMVSEPLEDLDPYYINKKTFIVLNKGKAI FRFSATSALYILTPFNPLRKIAIKILVHS
LFSMLIMCTILTNCVFMTMSNPPDWTKNVEYTFGTGIYTFESLIKIIARGFCLEDFTFLRDPWNW
LDFTVITFAYVTEFVDLGNVSALRTFRVLRALKTISVIPGLKTIVGALIQSVKKLSVDMILTVE
CLSVFALIGLQLFMGNLRNKCIQWPPTNASLEEHSIEKNITVNYNGTLINETVFEFDWKSIIQD
SRYHYFLEGFLDALLCGNSSDAGQCPEGYMCVKAGRNPNYGYTSFDTFSWAFLSLFRLMTQDFW
ENLYQLTLRAAGKTYMIFVFLVIFLGSFYLINLILAVVAMAYEEQNQATLEEAQEKEAEFQQMI
EQLKKQQEAAQQAATATASEHSREPSAAGRLSDSSSEASKLSSKSAKERRNRKRKRKQKEQSGG
EEKDEDEFQKSESEDSIRRKGFRRFSIEGNRLTYEKRYSSPHQSLLSIRGSLFSPRRNSRTSLFS
FRGRAKDVGSENFADDEHSTFEDNESRRDSL FVPRRHGERRNSNLSQTSRSSRMLAVFPANGK
MHSTVDCNGVVSLVGGPSVPTSPVGQLLPEVIIDKPATDDNGTTTETEMRKRRSSSFHVSMDFL
EDPSQRQRAMSIASILTNTVEELEESRQKCPCWYKFSNIFLIWDCSPYWLKVKHVVNLVVMDF
FVDLAITICIVLNTLFMAMEHYPMTDHFNNVLTVGNLVFTGIFTAEMFLKIIAMDPYFFFQEGW
NIFDGFIVTSLSLVELGLANVEGLSVLRSFRLLRVFKLAKSWPTLNMLIKIIGNSVGALGNLTLV
LAIIVFIFAVVGMQLFGKSYKDCVCKIASDCQLPRWHMNDFFHSFLIVFRVLCGEWIETMWDCM

EVAGQAMCLTVFMMVMVIGNLVVLNLFALALLSSFSADNLAATDDDNEMNNLQIAVDRMHKGVA
YVKRKIYEFIQQSFIRKQKILDEIKPLDDLNNKKDSCMSNHTAEIGKDL DY LKDVNGTTS GIGT
GSSVEKYIIDESDYMSFINNPSLTVTVPPIAVGESDFENLNTEDFSSES DLEESKEKLNES SSSS
EGSTVDIGAPVEEQPVVEPEETLEPEACFTEGCVQRFKCCQINVEEGRGKQWWNL RRTC FRIVE
5 HNW FET FIV FMILLSSGALAFEDIYIDQRKTIKTMLEYADKVFTYI FILEMLLKWVAYGYQTYF
TNAWCWLD FLIVDVSLVSLTANALGYSELGAIKSLRTLRLALRPLRALS RFEGMRVVVNALLGAI
PSIMNVLLVCLIFWLI FSIMGVNLFAGKFYHCINTTTGDRFDIEDVNNHTDCLKLIERNETARW
KNVKVNF DN VGFGYLSLLQVATFKGWMDIMYAAVDSRNVELQPKYEESLYMYLYFVIFII FGSF
FTLNLFIGVIIDNFNQKKKFGGQDIFMTEEQKKYYNAMKKLGSKKPQKPIPRPGNKFQGMVFD
10 FVTRQVFDISIMILICLNMVTMMVETDDQSEYVTTILSRINLVFIVLFTGECVLKLISLRHYF
TIGWNI FDFV VVILSIVGMFLAELIEKYFVSPTLFRVIRLARIGRILRLIKGAKGIRTLLFALM
MSLPALFNIGLLLFLVMFIYAI FGMSNFAYVKREVGIDDMFNFETFGNSMICLFQITTSAGWDG
LLAPILNSKPPDCDPNKVNP GSSVKGDCGNPSVGIFFFVSYIIISFLVVVNMYIAVILENFSVA
TEESAEP LSEDDFEMFYEVWEKFDPDATQFMFEKLSQFAAALEPPLNLPQPNKLQLIAMDLPM
15 VSGDRIHCLDILFAFTKRVLGESGEMDALRIQMEERFMASNP SKVSYQPITTTLKRKQEEVSAV
IIQRAYRRHLLKRTVKQASFTYNKNKIKGGANLLIKEDMIIDRINENSITEKTDLTMTAACPP
SYDRVTKPIVEKHEQEGKDEKAKGK (SEQ ID NO: 863);

MEQTVLVPPGPDSFNFFTRSLAAIERRIAEEKAKNP KPDKKDDDENGPKNSDLEAGKNLPFI
YGDIPPEMVSEPLEDLD PYYINKKTFIVLNKGKAI FRFSATSALYILTPFNPLRKIAIKILVHS
20 LFSMLIMCTILTNCVFM TMSNPPDWTKNVEYTF TGIYTFESLIKIIARGFCLEDFTFLRDPWNW
LDFTVITFAYVTEFVD LGNV SALRTFRVL RALKTISVIPGLKTIVGALIQSVKKLS DVMILT VF
CLSVFALIGLQLFMGNLRNKCIQWPPTNASLEEHSIEKNITVNYNGTLINETVFEFDWKS YIQD
SRYHYFLEGFLDALLCGNSSDAGQCPEGYMCVKAGRNP NYGYTSFDTFSWAFLSLFRLMTQDFW
ENLYQLTLRAAGKTYMIF FVLVI FLGSFYLINLILAVVAMAYEEQNQATLEAEQKEAEFQQMI
25 EQLKKQQEAAQQAATATASEHSREPSAAGRLSDSSSEASKLSSKSAKERRNRKRKRKQKEQSGG
EEKDEDEFQKSESEDSIRRKGF RF SIEGNRLTYEKRYSSPHQSLLSIRGSLFSPRRNSRTSLFS
FRGRAKDVGSENFADDEHSTFEDNESRRDSL FVPRRHGERRNSNLSQTSRSSRMLAVFPANGK
MHSTVDCNGVVSLVGGPSVPTSPVGQLLPEGTTTETEMRKRRSSSFHVSMDFLEDPSQRQRAMS
IASILTNTVEELEESRQKCPPCWYKFSNIFLIWDCSPYWLKVKHVVNLVMDPFVDLAITICIV
30 LNTLFMAMEHY PMTDHFNNVLT VGNLVFTGI FTAEMFLKIIAMDPYYY FQEGWNIFDGFIVTLS
LVELGLANVEGLSVLRSFRLLRVFKLAKSWPTLNMLIKI IGNSVGALGNLTLVLAIIVFI FAVV
GMQLFGKSYKDCVCKIASDCQLPRWHMNDFFHSFLIVFRVLCGEWIETMWD CMEVAGQAMCLTV
FMMVMVIGNLVVLNLFALALLSSFSADNLAATDDDNEMNNLQIAVDRMHKGVA YVKRKIYEFIQ
QSFIRKQKILDEIKPLDDLNNKKDSCMSNHTAEIGKDL DY LKDVNGTTS GIGTGSSVEKYIIDE

SDYMSFINNPSLTVTVPIAVGESDFENLNTEDFSSESLEESKEKLNESSSSSEGSTVDIGAPV
 EEQPVVEPEETLEPEACFTEGCVQRFKCCQINVEEGRGKQWWNLRRTCFRIVEHNWFETFIVFM
 ILLSSGALAFEDIYIDQRKTIKTMLEYADKVFTYIFILEMLLKWVAYGYQTYFTNAWCWLDFLI
 VDVSLVSLTANALGYSELGAIKSLRTLRLRPLRALS RFEGMRVVVNALLGAIPSIMNVLLVCL
 5 IFWLIFSIMGVNLFAGKFYHCINTTTGDRFDIEDVNNHTDCLKLIERNETARWKNVKVNFNVG
 FGYLSLLQVATFKGWMDIMYAAVDSRNVELQPKYEESLYMYLYFVIFIIFGSFFTLNLFIVGII
 DNFNQKKKKFGGQDIFMTEEQKKYYNAMKKLGSKKPQKPIPRPGNKFQGMVDFVTRQVFDISI
 MILICLNMVTMMVETDDQSEYVTTILSRINLVFIVLFTGECVLKLISLRHYYFTIGWNIFDFVV
 VILSIVGMFLAELIEKYFVSPTLFRVIRLARIGRIILRLIKGAKGIRTLFLALMMSLPALFNIGL
 10 LLFLVMFIYAI FGMSNFAYVKREVGIDDMFNFETFGNSMICLFQITTSAGWDGLLAPILNSKPP
 DCDPNKVNPGSSVKGDCGNPSVGIFFFVSYIIISFLVVNMYIAVILENFSVATEESAEPLESD
 DFEMFYEVWEKFDPDATQFMEFEKLSQFAAALEPPLNLPQPNKLQLIAMDLPMVSGDRIHCLDI
 LFAFTKRVLGESGEMDALRIQMEERFMASNPSKVSYPITTTTLKRKQEEVSAVIIQRAYRRHLL
 KRTVKQASFTYNKNKIKGGANLLIKEDMIIDRINENSITEKTDLTMTAACPPSYDRVTKPIVE
 15 KHEQEGKDEKAKGK (SEQ ID NO: 864);
 MEQTVLVPPGPDSFNFFTRSLAAIERRIAEEKAKNP KPDKKDDENGPKPNSDLEAGKNLPFI
 YGDIPPEMVSEPLEDLDPYYINKKTFIVLNKGKAI FRFSATSALYILTPFNPLRKIAIKILVHS
 LFSMLIMCTILTNCVFMTMSNPPDWTKNVEYTFGTIYTFESLIKIIARGFCLEDFTLRDPWNW
 LDFTVITFAYVTEFVDLGNVSALRTFRVLRLAKTISVIPGLKTIVGALIQSVKKLSVDMILTVF
 20 CLSVFALIGLQLFMGNLRNKCIQWPPTNASLEEHSIEKNITVNYNGTLINETVFEFDWKSXIQD
 SRYHYFLEGFLDALLCGNSSDAGQCPEGYMCVKAGRNPNYGYTSFDTFSWAFLSLFRLMTQDFW
 ENLYQLTLRAAGKTYMIFFVLVIFLGSFYLINLILAVVAMAYEEQNQATLEEAQEKEAEFQQMI
 EQLKKQQEAAQQAATATASEHSREPSAAGRLSDSSSEASKLSSKSAKERRNRKRKRKQKEQSGG
 EEKDEDEFQKSESEDSIRRKGF RFSEIEGNRLTYEKRYSSPHQSLLSIRGSLFSPRRNSRTSLFS
 25 FRGRAKDVGSENDFADDEHSTFEDNESRRDSL FVPRRHGERRNSNLSQTSRSSRMLAVFPANGK
 MHSTVDCNGVVSLGTTTETEMRKRRSSSFHVSMDFLEDPSQRQRAMSIIASILTNTVEELEESRQ
 KCPWCWKFSNIFLIWDCSPYWLKVKHVVNLVVMDFVVDLAITICIVLNTLFMAMEHYPMTDHF
 NNVLTVGNLVFTGIFTAEMFLKIIAMDPIYYYFQEGWNIFDGFIVTSLVELGLANVEGLSVLRS
 FRLLRVFKLAKSWPTLNMLIKIIGNSVGALGNLTLVLAIIVFIFAVVGMQLFGKSYKDCVCKIA
 30 SDCQLPRWHMNDFHHSFLIVFRVLCGEWIETMWDCMEVAGQAMCLTVFMMVMVIGNLVVLNLF
 ALLSSFSADNLAATDDDNEMNNLQIAVDRMHKGVAYVKRKIYEFIQQS FIRKQKILDEIKPLD
 DLNNKKDSCMSNHTAEIGKDL DYLKDVNGTTSIGIGTGSSVEKYIIDESDYMSFINNPSLTVTVP
 IAVGESDFENLNTEDFSSESLEESKEKLNESSSSSEGSTVDIGAPVEEQPVVEPEETLEPEAC
 FTEGCVQRFKCCQINVEEGRGKQWWNLRRTCFRIVEHNWFETFIVFMILLSSGALAFEDIYIDQ

RKTIKTMLEYADKVFTYI FILEMLLKWVAYGYQTYFTNAWCWLDFLIVDVSLVSLTANALGYSE
 LGAIKSLRTLRLALRPLRALS RFEGMRVVVNALLGAIPSIMNVLLVCLIFWLIFSIMGVNLFAGK
 FYHCINTTTGDRFDIEDVNNHTDCLKLIERNETARWKNVKVNFNDNVGFGYLSLLQVATFKGWMD
 IMYAAVDSRNVELQPKYEESLYMYLYFVIFIIFGSFFTLNLFIGVIIDNFNQQKKKFGGQDIFM
 5 TEEQKKYYNAMKKLGSKKPQKPIPRPGNKFQGMVFDFVTRQVFDISIMILICLNMVTMMVETDD
 QSEYVTTILSRINLVFIVLFTGECVLKLISLRHYFTIGWNIFDFVVLISIVGMFLAELIEKY
 FVSPTLFRVIRLARIGRILRLIKGAKGIRTLLFALMMSLPALFNIGLLLFLVMFIYAI FGMSNF
 AYVKREVGIDDMFNFETFGNSMICLFQITTSAGWDGLLAPILNSKPPDCDPNKVNPGSSSVKGDC
 GNPSVGIFFFVSYIIISFLVVVNMYIAVILENFSVATEESAEPLEDDFEMFYEVEWEKFDPDAT
 10 QFMFEKLSQFAAALEPPLNLPQPNKLQLIAMDLPMVSGDRIHCLDILFAFTKRVLGESGEMDA
 LRIQMEERFMASNPSKVSYPITTTLKRKQEEVSAVIIQRAYRRHLLKRTVKQASFTYNKNKIK
 GGANLLIKEDMIIDRINENSITEKTDLTMTAACPPSYDRVTKPIVEKHEQEGKDEKAKGK

(SEQ ID NO: 865);

MEQTVLVPPGPDSFNFFTRSLAAIERRIAEEKAKNP KPDKKDDDENGPKPNSDLEAGKNLPFI
 15 YGDIPPEMVSEPLEDLDPYINKKTFIVLNKGKAI FRFSATSALYILTPFNPLRKIAIKILVHS
 LFSMLIMCTILTNCVFM TMSNPPDWTKNVEYFTFTGIYTFESLIKIIARGFCLEDFTLRDPWNW
 LDFTVITFAYVTEFVDLGNVSALRTFRVLRALKTISVIPGLKTIVGALIQSVKKLS DVMILTVE
 CLSVFALIGLQLFMGNLRNKCIQWPPTNASLEEHSIEKNITVNYNGTLINETVFEFDWKS YIQD
 SRYHYFLEGFLDALLCGNSSDAGQCPEGYMCVKAGRNP NYGYTSFDTFSWAFLSLFRLMTQDFW
 20 ENLYQLTLRAAGKTYMIF FVLVIFLG SFYLINLILAVVAMAYEEQNQATLEEA EQKEAEFQQMI
 EQLKKQQEAAQAATATASEHSREPSAAGRLSDSSSEASKLSSKSAKERRNRRKKRKQKEQSGGE
 EKDEDEFQKSESEDSIRRKGRFSIEGNRLTYEKRYSSPHQSLLSIRGSLFSPRNSRTSLFSF
 RGRAKDVGSENFADDEHSTFEDNESRRDSL FVPRRHGERRNSNLSQTSRSSRMLAVFPANGKM
 HSTVDCNGVVSLVGGPSVPTSPVGQLLPEGTTTETEMRKRRSSSFHVSMDFLEDPSQRQRAMSI
 25 ASILTNTVEELEESRQKCPWCYKFSNIFLIWDCSPYWLKVKHVVNLVVMDFVVDLAITICIVL
 NTLFMAMEHYPM TDHFNNVLT VGNLVFTGIFTAEMFLKIIAMDPYYYFQEGWNIFDGFIVTSL
 VELGLANVEGLSVLRSFRLLRVFKLAKSWPTLNMLIKIIGNSVGALGNLTLVLAIIVFIFAVVG
 MQLFGKSYKDCVCKIASDCQLPRWHMNDFFHSFLIVFRVLCGEWIETMWDCMEVAGQAMCLTVF
 MMVMVIGNLVVLNLF LALLSSFSADNLAATDDD NEMNNLQIAVDRMHKGVAYVKRKIYEFIQQ
 30 SFIRKQKILDEIKPLDDLNNKKDSCMSNHTAEIGKDL DYLDVNGTTSGIGTGSSVEKYIIDES
 DYMSFINNPSLT VTVPIAVGESDFENLNTEDFSSES DLEESKEKLNESSSSSEGSTVDIGAPVE
 EQPVVEPEETLEPEACFTEGCVQRFKCCQINVEEGRGKQWWNLRRTCFRIVEHNWFETFIVFMI
 LLSSGALAFEDIYIDQRKTIKTMLEYADKVFTYI FILEMLLKWVAYGYQTYFTNAWCWLDFLIV
 DVSLVSLTANALGYSELGAIKSLRTLRLALRPLRALS RFEGMRVVVNALLGAIPSIMNVLLVCLIF
 35 FWLIFSIMGVNLFAGKFYHCINTTTGDRFDIEDVNNHTDCLKLIERNETARWKNVKVNFNDNVGF

GYLSLLQVATFKGWMDIMYAAVDSRNVELQPKYEESLYMYLYFVIFIIFGSFFTLNLFIVGVIID
 NFNQQKKKFGGQDIFMTEEQKKYYNAMKKLGSKKPQKPIPRPGNKFQGMVFDFVTRQVFDISIM
 ILICLNMVTMMVETDDQSEYVTTILSRINLVFIVLFTGECVLKLISLRHYYFTIGWNI FDFV VV
 ILSIVGMFLAELIEKYFVSPTLFRVIRLARIGRILRLIKGAKGIRTLLFALMMSLPALFNIGLL
 5 LFLVMFIYAI FGMSNFAYVKREVGIDDMFN FETFGNSMICLFQITTSAGWDGLLAPILNSKPPD
 CDPNKVNPGSSVKGDCGNPSVGIFFFVSYIIISFLVVVNMYIAVILENFSVATEESAEPLEDD
 FEMFYEVEWEKFDPDATQFMEFEKLSQFAAALEPPLNLPQPNKLQLIAMDLPMVSGDRIHCLDIL
 FAFTKRVLGESGEMDALRIQMEERFMASNPSKVSYPITTTTLKRKQEEVSAV I IQRAYRRHLLK
 RTVKQASFTYNKNKIKGGANLLIKEDMIIDRINENSITEKTDLTMTAACPPSYDRVTKPIVEK
 10 HEQEGKDEKAKGK (SEQ ID NO: 866);
 MEQTVLVPPGPDSFNFFTRESLAAIERRIAEEKAKNP KPDKKDDDENGPKPNSDLEAGKNLPFI
 YGDIPPEMVSEPLEDLDPYYINKKTFIVLNKGKAI FRFSATSALYILTPFNPLRKIAIKILVHS
 LFSMLIMCTILTNCVFM TMSNPPDWTKNVEYTF TGIYTFESLIKIIARGFCLEDFTFLRDPWNW
 LDFTVITFAYVTEFVDLGNVSALRTFRVL RALKTISVIPGLKTIVGALIQSVKKLS DVMILT VF
 15 CLSVFALIGLQLFMGNLRNKCIQWPPTNASLEEHSIEKNITVNYNGTLINETVFEFDWKS YIQD
 SRYHYFLEGFLDALLCGNSSDAGQCPEGYMCVKAGRNP NYGYTSFDTFSWAFLSLFRLMTQDFW
 ENLYQLTLRAAGKTYMIF FVLVIFLGSFYLINLILAVVAMAYEEQNQATLEEA EQKEAEFQQMI
 EQLKKQQEAAQAATATASEHSREPSAAGRLSDSSSEASKLSSKSAKERRNRKRKRKQKEQSGGE
 EKDEDEFQKSESEDSIRRKGRFSIEGNRLTYEKRYSSPHQSLLSIRGSLFSPRRNSRTSLFSF
 20 RGRAKDVGSEND FADDEHSTFEDNESRRDSL FVPRRHGERRNSNLSQTSRSSRMLAVFPANGKM
 HSTVDCNGVVSLGTTTTETEMRKRRSSSFHVSMDFLEDPSQRQRAMS IASILTNTVEELEESRQK
 CPPCWYKFSNIFLIWDCSPYWLKVKHVVNLVVMDFVDLAITICIVLNTLFMAMEHYPM TDHFN
 NVLTVGNLVFTGIFTAEMFLKIIAMDPYYYFQEGWNI FDGFIVT LSLVELGLANVEGLSVLRSF
 RLLRVFKLAKSWPTLNMLIKIIGNSVGALGNLTLVLAIIVFIFAVVGMQLFGKSYKDCVCKIAS
 25 DCQLPRWHMNDFFHSFLIVFRVLCGEWIETMWDCEVAGQAMCLTVFMMVMVIGNLVVLNLFLA
 LLLSSFSADNLAATDDD NEMNNLQIAVDRMHKGVAYVKRKIYE FIIQQSFIRKQKILDEIKPLDD
 LNNKKDSCMSNHTAEIGKDL DYLKDVNGTTS GIGTGSSVEKYIIDESDYMSFINNPSLT VTVPI
 AVGESDFENLNTEDFSSES DLEESKEKLNESSSSSEGSTVDIGAPVEEQPVVEPEETLEPEACF
 TEGCVQRFKCCQINVEEGRGKQWWNL RRTCFRIVEHNWFET FIVFMILLSSGALAFEDIYIDQR
 30 KTIKTMLEYADKVFTYIFILEMLLKWVAYGYQTYFTNAWCWLD FLIVDVSLVSLTANALGYSEL
 GAIKSLRTL RALRPLRALS RFEGMRVVVNALLGAI PSIMNVLLVCLIFWLI FSIMGVNLFAGKF
 YHCINTTTGDRFDIEDVNNHTDCLKLIERNETARWKNVKNVFDNVGFGYLSLLQVATFKGWMDI
 MYAAVDSRNVELQPKYEESLYMYLYFVIFIIFGSFFTLNLFIVGVIIDN FNQQKKKFGGQDIFMT
 EEQKKYYNAMKKLGSKKPQKPIPRPGNKFQGMVFDFVTRQVFDISIMILICLNMVTMMVETDDQ

SEYVTTILSRINLVFIVLFTGECVLKLISLRHYYFTIGWNI FDFV VVILSIVGMFLAELIEKYF
 VSPTLFRVIRLARIGRILRLIKGAKGIRTLLFALMMSLPALFNIGLLLFLVMFIYAI FGMSNFA
 YVKREVGIDDMFNFETFGNSMICLFQITTSAGWDGLLAPILNSKPPDCDPNKVNP GSSVKGDCG
 NPSVGIFFFVSYIIISFLVVVNMYIAVILENFSVATEESAEP LSEDDFEMFYEVWEKFDPDATQ
 5 FMEFEKLSQFAAALEPPLNLPQPNKLQLIAMDLPMVSGDRIHCLDILFAFTKRVLGESGEMDAL
 RIQMEERFMASNP SKVSYQPITTTLKRKQEEVSAV I IQRAYRRHLLKRTVKQASFTYNKNKIKG
 GANLLIKEDMI IDRINENSITEKTDLTMTAACPPSYDRVT KPIVEKHEQEGKDEKAKGK (SEQ
 ID NO: 867); or

MFLKIIAMDPYYYFQEGWNI FDGFIVTSLVELGLANVEGLSVLRSFRLLRVFKLAKSWPTLNM
 10 LIKIIGNSVGALGNLTLVLAIIVFI FAVVGMQLFGKSYKDCVCKIASDCQLPRWHMNDFH SFL
 IVFRVLCGEWIE TMWDCMEVAGQAMCLTVFMMVMVIGNLVVLNLF LALLSSFSADNLAATDDD
 NEMNNLQIAVDRMHKGVAYVKRKIYEFIQQS FIRKQKILDEIKPLDDLNNKKDSCMSNHTAEIG
 KDL DYLKDVNGTTS GIGTGSSVEKYIIDESDYMSFINNPSLTVTVPIAVGESDFENLNTEDFSS
 ESDLEESKEKLNESSSSSEGSTVDIGAPVEEQPVVEPEETLEPEACFTEGCVQRFKCCQINVEE
 15 GRGKQWWNLRRTCFRIVEHNWFETFIVFMILLSSGALAFEDIYIDQRKTIKTMLEYADKVFTYI
 FILEMLLKWVAYGYQTYFTNAWCWLDFLIVDVSLVSLTANALGYSELGAIKSLRTLRLALRPLRA
 LSRFEGMRVVVNALLGAI PSIMNVLLVCLIFWLI FSIMGVNLFAGKFYHCINTTTGDRFDIEDV
 NNHTDCLKLIERNETARWKNVKVNFDNVGFGYLSLLQVATFKGWMDIMYAAVDSRNVELQPKYE
 ESLYMYLYFVIFII FGSFFTLNLFIGVIIDNFNQQKKKFGGQDIFMTEEQKKYYNAMKKLGSKK
 20 PQKPIPRPGNKFQGMVFDFVTRQVFDISIMILICLNMVTMMVETDDQSEYVTTILSRINLVFIV
 LFTGECVLKLISLRHYYFTIGWNI FDFV VVILSIVGMFLAELIEKYFVSPTLFRVIRLARIGRI
 LRLIKGAKGIRTLLFALMMSLPALFNIGLLLFLVMFIYAI FGMSNFA YVKREVGIDDMFNFET F
 GNSMICLFQITTSAGWDGLLAPILNSKPPDCDPNKVNP GSSVKGDCGNPSVGIFFFVSYIIISF
 LVVVNMYIAVILENFSVATEESAEP LSEDDFEMFYEVWEKFDPDATQFMEFEKLSQFAAALEPP
 25 LNL PPNKLQLIAMDLPMVSGDRIHCLDILFAFTKRVLGESGEMDALRIQMEERFMASNP SKVS
 YQPITTTLKRKQEEVSAV I IQRAYRRHLLKRTVKQASFTYNKNKIKG GANLLIKEDMI IDRINE
 NSITEKTDLTMTAACPPSYDRVT KPIVEKHEQEGKDEKAKGK (SEQ ID NO: 868).

[00171] The term “effective amount” as used herein refers to the amount of an active agent
 (e.g., tRNA or expression vector according to the present invention or a secondary active agent
 30 in a combination therapy) sufficient to effect beneficial or desired results. An effective amount
 can be administered in one or more administrations, applications or dosages and is not intended
 to be limited to a particular formulation or administration route.

[00172] As used herein, “treat”, “treating” and “treatment” mean the treatment of a disease in a
 subject, e.g., in a human. This includes: (a) inhibiting the disease, *i.e.*, arresting its

development; and (b) relieving the disease, *i.e.*, causing regression of the disease state. As used herein, the terms “subject” and “patient” refer to an organism to be treated by the methods and compositions described herein. Such organisms preferably include, but are not limited to, mammals (*e.g.*, murines, simians, equines, bovines, porcines, canines, felines, and the like), and
5 more preferably includes humans.

[00173] The methods and compositions described herein can be used alone or in combination with other therapeutic agents and/or modalities. The term administered “in combination,” as used herein, is understood to mean that two (or more) different treatments are delivered to the subject during the course of the subject’s affliction with the disorder, such that the effects of the
10 treatments on the patient overlap at a point in time. In certain embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as “simultaneous” or “concurrent delivery.” In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In certain embodiments of either case, the treatment is more effective
15 because of combined administration. For example, the second treatment is more effective, *e.g.*, an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In certain embodiments, delivery is such that the reduction in a symptom, or other parameter
20 related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

[00174] In certain embodiments, a method or composition described herein is administered in
25 combination with one or more additional therapeutic agents, *e.g.*, DIACOMIT[®] (stiripentol), EPIODOLEX[®] (cannabidiol), a ketogenic diet, ONFI[®] (clobazam), TOPAMAX[®] (topiramate), fenfluramine, or valproic acid. For example, during the treatment of Dravet Syndrome, a method or composition described herein is administered in combination with one or more additional therapeutic agents, *e.g.*, DIACOMIT[®] (stiripentol), EPIODOLEX[®] (cannabidiol), a
30 ketogenic diet, ONFI[®] (clobazam), TOPAMAX[®] (topiramate), fenfluramine, or valproic acid.

[00175] Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are

compositions of the present invention that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing steps.

5 [00176] In the application, where an element or component is said to be included in and/or selected from a list of recited elements or components, it should be understood that the element or component can be any one of the recited elements or components, or the element or component can be selected from a group consisting of two or more of the recited elements or components.

10 [00177] Further, it should be understood that elements and/or features of a composition or a method described herein can be combined in a variety of ways without departing from the spirit and scope of the present invention, whether explicit or implicit herein. For example, where reference is made to a particular compound, that compound can be used in various embodiments of compositions of the present invention and/or in methods of the present invention, unless otherwise understood from the context. In other words, within this application, embodiments
15 have been described and depicted in a way that enables a clear and concise application to be written and drawn, but it is intended and will be appreciated that embodiments may be variously combined or separated without parting from the present teachings and invention(s). For example, it will be appreciated that all features described and depicted herein can be applicable to all aspects of the invention(s) described and depicted herein.

20 [00178] It should be understood that the expression “at least one of” includes individually each of the recited objects after the expression and the various combinations of two or more of the recited objects unless otherwise understood from the context and use. The expression “and/or” in connection with three or more recited objects should be understood to have the same meaning unless otherwise understood from the context.

25 [00179] The use of the term “include,” “includes,” “including,” “have,” “has,” “having,” “contain,” “contains,” or “containing,” including grammatical equivalents thereof, should be understood generally as open-ended and non-limiting, for example, not excluding additional unrecited elements or steps, unless otherwise specifically stated or understood from the context.

30 [00180] Where the use of the term “about” is before a quantitative value, the present invention also includes the specific quantitative value itself, unless specifically stated otherwise. As used herein, the term “about” refers to a $\pm 10\%$ variation from the nominal value unless otherwise indicated or inferred.

[00181] It should be understood that the order of steps or order for performing certain actions is immaterial so long as the present invention remain operable. Moreover, two or more steps or actions may be conducted simultaneously.

[00182] The use of any and all examples, or exemplary language herein, for example, “such as” or “including,” is intended merely to illustrate better the present invention and does not pose a limitation on the scope of the invention unless claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the present invention.

EXAMPLES

[00183] The following Examples are merely illustrative and are not intended to limit the scope or content of the invention in any way.

Example 1

[00184] This Example describes arginine aminoacylated suppressor tRNAs that facilitate read-through of a premature termination codon (PTC).

[00185] Suppressor tRNAs were generated from endogenous mouse arginine-tRNAs by converting their normal anticodons to TCA anticodons that recognize TGA termination codons (referred to as Arg_{TCA} suppressor tRNAs). Five of the endogenous arginine-tRNAs contained introns that must be spliced out in order to produce mature tRNAs; corresponding Arg_{TCA} suppressor tRNAs with and without these intronic sequences were generated. Suppressor tRNA sequences are shown in TABLE 8.

TABLE 8

SEQ ID NO	Suppressor tRNA ID Number	Suppressor tRNA Name	Suppressor tRNA Sequence (anticodon lowercase)
1	089	tRNA-Arg-ACG-1-1-TCA-SUP	GGGCCAGTGGCGCAATGGATAACG CGTCTGACTtcaGATCAGAAGATTTC CAGGTTTCGACTCCTGGCTGGCTCG
2	090	tRNA-Arg-ACG-2-1-TCA-SUP	GGGCCAGTGGCGCAATGGATAACG CGTCTGACTtcaGATCAGAAGATTG TAGGTTTCGACTCCTACCTGGCTCG

3	101	tRNA-Arg-ACG-3-1-TCA-SUP	GGGCCAGTGGCGCAATGGATAACG CGTCTGACTtcaGATCAGAAGATTCTAGGTTTCGACTCCTGGCTGGCTCG
4	102	tRNA-Arg-CCG-1-1-TCA-SUP	GGCCGCGTGGCCTAATGGATAAGG CGTCTGATTtcaGATCAGAAGATTGAGGGTTTCGAGTCCCTTCGTGGTTCG
5	103	tRNA-Arg-CCG-2-1-TCA-SUP	GGCCGCGTGGCCTAATGGATAAGG CGTCTGATTtcaGATCAGAAGATTGGGGTTTCGAGTCCCTTCGTGGTTCG
6	104	tRNA-Arg-CCG-3-1-TCA-SUP	GACCCAGTGGCCTAATGGATAAGG CATCAGCCTtcaGAGCTGGGGATTGTGGGTTTCGAGTCCCACCTCTGGGTTCG
7	105	tRNA-Arg-CCT-1-1-TCA-SUP	GCCCCAGTGGCCTAATGGATAAGG CACTGGCCTtcaAAGCCAGGGATTGTGGGTTTCGAGTCCCACCTGGGGTA
8	106	tRNA-Arg-CCT-2-1-TCA-SUP	GCCCCAGTGGCCTAATGGATAAGG CACTGGCCTtcaAAGCCAGGGATTGTGGGTTTCGAGTCCCACCTGGGGTG
9	107	tRNA-Arg-CCT-3-1-TCA-SUP	GCCCCGGTGGCCTAATGGATAAGGC ATTGGCCTtcaAAGCCAGGGATTGTGGTTTCGAGTCCCACCCGGGGTA
10	108	tRNA-Arg-CCT-4-1-TCA-SUP	GCCCCAGTGGCCTAATGGATAAGGC ATTGGCCTtcaAAGCCAGGGATTGTGGTTTCGAGTCCCACCTCTGGGGTG
11	001	tRNA-Arg-TCG-1-1-TCA-SUP	GGCCGCGTGGCCTAATGGATAAGGC GTCTGACTtcaGATCAGAAGATTGCA GGTTTCGAGTCCTGCCGCGGTTCG
12	109	tRNA-Arg-TCG-2-1-TCA-SUP	GACCGCGTGGCCTAATGGATAAGGC GTCTGACTtcaGATCAGAAGATTGAGGGTTTCGAGTCCCTTCGTGGTTCG
13	110	tRNA-Arg-TCG-3-1-TCA-SUP	GACCACGTGGCCTAATGGATAAGGC GTCTGACTtcaGATCAGAAGATTGAGGGTTTGAATCCCTTCGTGGTTG

14	111	tRNA-Arg-TCG-4-1-TCA-SUP	GACCACGTGGCCTAACGGATAAGGC GTCTGACTtcaGATCAGAAGATTGAG GGTTCGAATCCCTTCGTGGTTA
15	112	tRNA-Arg-TCT-1-1-TCA-SUP_contains intron	GGCTCTGTGGCGCAATGGATAGCGC ATTGGACTtcaAGTGACGAGAAAGCG ATTCAAAGGTTGTGGGTTCGAATCCC ACCAGAGTCG
16	113	tRNA-Arg-TCT-1-1-TCA-SUP_no intron	GGCTCTGTGGCGCAATGGATAGCGC ATTGGACTtcaAATTCAAAGGTTGTG GGTTCGAATCCCACCAGAGTCG
17	114	tRNA-Arg-TCT-2-1-TCA-SUP_contains intron	GGCTCCGTGGCGCAATGGATAGCGC ATTGGACTtcaAGAGGCTGAAGGCAT TCAAAGGTTCCGGGTTCGAGTCCCGG CGGAGTCG
18	115	tRNA-Arg-TCT-2-1-TCA-SUP_no intron	GGCTCCGTGGCGCAATGGATAGCGC ATTGGACTtcaAATTCAAAGGTTCCG GGTTCGAGTCCCGGCGGAGTCG
19	116	tRNA-Arg-TCT-3-1-TCA-SUP_contains intron	GGCTCTGTGGCGCAATGGATAGCGC ATTGGACTtcaAGCATGATTGAGAGA TTCAAAGGTTGCGGGTTCGAGTCCCG CCAGAGTCG
20	117	tRNA-Arg-TCT-3-1-TCA-SUP_no intron	GGCTCTGTGGCGCAATGGATAGCGC ATTGGACTtcaAATTCAAAGGTTGCG GGTTCGAGTCCCGCCAGAGTCG
21	118	tRNA-Arg-TCT-4-1-TCA-SUP_contains intron	GGCTCTGTGGCGCAATGGATAGCGC ATTGGACTtcaAGACAAATGGAGGCA TTCAAAGGTTGTGGGTTCGAGTCCCA CCAGAGTCG
22	119	tRNA-Arg-TCT-4-1-TCA-SUP_no intron	GGCTCTGTGGCGCAATGGATAGCGC ATTGGACTtcaAATTCAAAGGTTGTG GGTTCGAGTCCCACCAGAGTCG
23	120	tRNA-Arg-TCT-5-1-TCA-SUP	GTCTCTGTGGCGCAATGGACGAGCG CGCTGGACTtcaAATCCAGAGGTTCT GGGTTCGAGTCCCGGCAGAGATG

24	121	tRNA-Arg-TCT-6-1-TCA-SUP_contains intron	GGCTCTGTGGAGCAATGGATAGCAC ATTGGACTtcaAGCATGACCGAGAGA TTCAAAGGTTGCGGGTTCGAGTCCCA CCAGAGTTG
25	122	tRNA-Arg-TCT-6-1-TCA-SUP_no intron	GGCTCTGTGGAGCAATGGATAGCAC ATTGGACTtcaAATTCAAAGGTTGCG GGTTCGAGTCCCACCAGAGTTG
35	179	tRNA-Arg-TCT-5-1-TCA-SUP_T51C	GTCTCTGTGGCGCAATGGACGAGCG CGCTGGACTtcaAATCCAGAGGTTCC GGGTTTCGAGTCCCGGCAGAGATG

[00186] In this Example, all mature tRNA sequences (as predicted by GtRNAdb; <http://gtrnadb.ucsc.edu>) were expressed in the context of upstream and downstream genomic flanking sequences (± 200 bps) from tRNA-Arg-TCG-1-1 — a highly expressed arginine-tRNA, i.e., the tRNA sequences were expressed with a 5' flanking sequence of SEQ ID NO: 26 and a 3' flanking sequence of SEQ ID NO: 27. All mature tRNA sequences including upstream and downstream genomic flanking sequences were generated in a pGL4 vector backbone.

[00187] The ArgTCA suppressors were tested for PTC readthrough activity by flow cytometry in cell lines containing dual fluorescent readthrough reporters. These reporters include three copies of a red fluorescent protein (tdTomato), TEV protease, a linker region containing a PTC, and three copies of a green fluorescent protein (EGFP). A schematic of a reporter construct is shown in FIGURE 3. In the absence of any PTC readthrough as a result of a suppressor tRNA, translation will be terminated by the PTC within the linker region, and only tdTomato will be expressed (and therefore only red fluorescence detected). PTC readthrough activity as a result of a suppressor tRNA will allow translation to proceed through the PTC in the linker region, and for both tdTomato and EGFP to be expressed (and therefore both red and green fluorescence detected). Accordingly, readthrough can be assessed by quantifying the percentage of viable cells expressing both the red and green fluorescent reporters above background (double positive %).

[00188] To screen for suppressor tRNAs with readthrough activity at PTCs relevant to Dravet syndrome, linker regions were generated containing the PTC and eight flanking codons on either side of the PTC from the SCN1A transcript of two patients with nonsense mutations in the SCN1A gene: subject N and subject S.

[00189] The linker region derived from the subject N SCN1A transcript is as follows, and the reporter including this linker region is referred to as the subject N-PTC reporter:

CTGAGACCTCTAAGAGCCTTATCTtgaTTTGAAGGGATGAGGGTGGTTGTG (SEQ ID NO: 28).

A corresponding linker with a wild-type Arg codon in place of the PTC was used as a control, and had the following sequence:

CTGAGACCTCTAAGAGCCTTATCTcgaTTTGAAGGGATGAGGGTGGTTGTG (SEQ ID NO: 193).

[00190] The linker region derived from the subject S SCN1A transcript is as follows, and the reporter including this linker region is referred to as the subject S-PTC reporter:

ACAAGCCTTTTCAGCTTTAGAGGGtgaGCAAAGGATGTGGGATCTGAGAAC (SEQ ID NO: 29).

A corresponding linker with a wild-type Arg codon in place of the PTC was used as a control, and had the following sequence:

ACAAGCCTTTTCAGCTTTAGAGGGcgaGCAAAGGATGTGGGATCTGAGAAC (SEQ ID NO: 194).

[00191] An additional 51 base pair linker region was derived from a mouse model of Dravet syndrome caused by an R1407X nonsense mutation in SCN1A (Ogiwara et al., 2007, Neurobiology of Disease). The linker region derived from the SCN1A R1407X transcript is as follows, and the reporter including this linker region is referred to as R1407X-PTC reporter:

CTAATAGAAAGAAATGAGACCGCctgaTGGAAAAATGTGAAAGTAACTTT (SEQ ID NO: 30).

A corresponding linker with a wild-type Arg codon in place of the PTC was used as a control, and had the following sequence:

CTAATAGAAAGAAATGAGACCGCCcggTGGAAAAATGTGAAAGTAACTTT (SEQ ID NO: 195).

[00192] Arg_{TCA} suppressors were tested in multiple assay contexts, including (i) a human Flp-In-293 cell line stably expressing the subject S-PTC reporter and transiently transfected with a plasmid encoding an Arg_{TCA} suppressor (results shown in **FIGURE 4**), (ii) a murine Flp-In-3T3 cell line stably expressing the R1407X-PTC reporter and transiently transfected with a plasmid encoding an Arg_{TCA} suppressor (results shown in **FIGURE 5**), and (iii) Flp-In-293 cells transiently co-transfected with plasmids encoding the subject N-PTC reporter and an Arg_{TCA} suppressor tRNA (results shown in **FIGURE 6**). Transfections were done using the Lipofectamine 3000 Transfection Reagent according to the manufacturer's protocol.

[00193] An additional reporter construct was generated including EGFP with a nuclear localization signal (NLS) and containing an arginine-to-TGA mutation (R96X) within the EGFP open reading frame that abolishes fluorescence in the absence of PTC readthrough. The general experimental approach is shown in **FIGURE 8A**. EGFP expression was driven by the CMV early enhancer/chicken β actin (CAG) promoter. The reporter construct is referred to as CAG:NLS-EGFP (R96X-TGA) and its sequence is as follows:

CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTTGACG
TCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGG
AGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCC
10 TATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGAC
TTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCA
CGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTT
TTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGG
CGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGC
15 TCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCG
GCGGGCGGGAGTCGCTGCGCGCTGCCTTCGCCCCGTGCCCCGCTCCGCCGCCGCCTCGCGCCGC
CCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTC
CGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTGCGTGAAAGCCT
TGAGGGGCTCCGGGAGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCG
20 CCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGG
GGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACC
ATGTTTCATGCCTTCTTCTTTTCTTCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCA
TCATTTTGGCAAAGAATTGCGGCCCAACGGTACCGGATCCACCGGCCGCCACCATGGGAAGCCC
AAAGAAGAAGCGTAAGGTAATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATC
25 CTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCG
ATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCCGTGCCCTG
GCCACCCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATG
AAGCAGCACGACTTCTTCAAGTCCGCCATGCCCCGAAGGCTACGTCCAGGAGTGAACCATCTTCT
TCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAA
30 CCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAG
TACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGA
ACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAA
CACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCC
CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCG

GGATCACTCTCGGCATGGACGAGCTGTACAAGGGAAGCCCCAAGAAAAAGCGGAAGGTGTAA
(SEQ ID NO: 31).

[00194] The activity of the Arg^{TCA} suppressors was assessed in HEK293 cells and murine Neuro-2a cells (a neural crest-derived cell line extensively used to study neuronal differentiation) transiently co-transfected with a plasmid encoding the Arg^{TCA} suppressor tRNA and a plasmid encoding the CAG:NLS-EGFP (R96X-TGA) reporter. Transfections were done using the Lipofectamine 3000 Transfection Reagent according to the manufacturer's protocol. Co-transfections were done using equal amounts of the indicated suppressor plasmid and the CAG:NLS-EGFP (R96X-TGA) reporter plasmid. EGFP expression was analyzed by flow cytometry at ~24 hours post transfection in 293 cells and ~48 hours post transfection in Neuro-2a cells. The results are shown in **FIGURE 7**.

[00195] In general, the relative readthrough activity of Arg^{TCA} suppressor tRNAs remained consistent across multiple assay formats. The following suppressors reliably showed readthrough activity above baseline: TCA-001 (SEQ ID NO: 11), TCA-89 (SEQ ID NO: 1), TCA-90 (SEQ ID NO: 2), TCA-105 (SEQ ID NO: 7), TCA-106 (SEQ ID NO: 8), TCA-107 (SEQ ID NO: 9), TCA-113 (SEQ ID NO: 16), TCA-114 (SEQ ID NO: 17), TCA-115 (SEQ ID NO: 18), TCA-116 (SEQ ID NO: 19), TCA-117 (SEQ ID NO: 20), TCA-118 (SEQ ID NO: 21), and TCA-119 (SEQ ID NO: 22).

[00196] Together, these results demonstrate that the described suppressor tRNAs can facilitate expression of transcripts, *e.g.*, SCN1A transcripts, containing premature termination codons associated with disorders, *e.g.*, Dravet syndrome.

Example 2

[00197] This Example describes the impact of expression vector features on the read-through of a premature termination codon (PTC) by arginine aminoacylated suppressor tRNAs.

[00198] Expression constructs containing both Arg^{TCA} suppressor tRNAs and EGFP (R96X-TGA) reporters on the same plasmid were generated in a pGL4 vector backbone. These constructs included 1, 2, 3 or 4 copies of Arg^{TCA} suppressor tRNAs 113 (SEQ ID NO: 16), 115 (SEQ ID NO: 18), and 001 (SEQ ID NO: 11) (as described in Example 1 and shown in **TABLE 8**). Each copy of the tRNA sequences was expressed in the context of either (i) 200 bps upstream genomic flanking sequences from tRNA-Arg-TCG-1-1 (SEQ ID NO: 26) and 200 bps of downstream genomic flanking sequence from tRNA-Arg-TCG-1-1 (SEQ ID NO: 27), (ii) 200 bps upstream genomic flanking sequences from tRNA-Arg-TCG-1-1 (SEQ ID NO: 26) and 104 bps of downstream genomic flanking sequence from tRNA-Arg-TCG-1-1 (SEQ ID NO: 32), or

(iii) an upstream U6 promoter including 19 bps of upstream flanking genomic sequence from tRNA-Arg-TCG-1-1 (SEQ ID NO: 33) and 46 bps of downstream flanking genomic sequence from tRNA-Arg-TCG-1-1 (SEQ ID NO: 34). The general experimental approach is shown in **FIGURE 8A** and a schematic of an exemplary reporter construct containing 4 copies of the suppressor tRNA is shown in **FIGURE 8B**. Reporter constructs were either a CAG:NLS-EGFP (R96X-TGA) reporter construct (as described in Example 1) or an EF1a:NLS-EGFP (R96X-TGA) reporter construct in which the CAG promoter was replaced with an elongation factor-1 alpha (EF1a) promoter, whose sequence is as follows:

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GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAG
10 GGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGT
GTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCACTAGTCGCCGTG
AACGTTCTTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCG
GGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGTA
CGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTTCGTGGCCTTGCGCTTAA
15 GGAGCCCCTTCGCCTCGTGCTTGAGTTGTGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAA
TCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTGTG
ATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAATGCGGGCCAAGATCAGCAC
ACTGGTATTTTCGGTTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTT
CGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGCCCG
20 GCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCC
CGGTCGGCACCAAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCACAA
AATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTT
TCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGAT
TAGTTCTCCAGCTTTTGGAGTACGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGT
25 TTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTT
GGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGT
TTTTTTCTTCCATTTCAAGTGTCGTGAGGTACCGGATCCACCGGCCGCCACCATGGGAAGCCCA
AAGAAGAAGCGTAAGGTAATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCC
TGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAAGCGTGTCCGGCGAGGGCGAGGGCGA
30 TGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCCTGCCCTGG
CCCACCCTCGTGACCACCCTGACCTACGGCGTGCAAGTCTTCAAGCGCTACCCCGACCACATGA
AGCAGCACGACTTCTTCAAGTCCGCCATGCCCCGAAGGCTACGTCCAGGAGTGAACCATCTTCTT
CAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAAC
CGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGT

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ACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAA
 CTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAAC
 ACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCCGACAACCACTACCTGAGCACCCAGTCCGCCC
 TGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGG
 5 GATCACTCTCGGCATGGACGAGCTGTACAAGGGAAGCCCCAAGAAAAAGCGGAAGGTGTAA
 (SEQ ID NO: 177).

[00199] Neuro-2a cells or HEK293 (FlpIn-293) cells were transfected with these constructs and readthrough was assayed by fluorescent imaging or flow cytometry ~24 hours or 48 hours post transfection. Transfections were done using the Lipofectamine 3000 Transfection Reagent
 10 according to the manufacturer's protocol.

[00200] The results are shown **FIGUREs 9-10** (fluorescent images) and **FIGUREs 11-17** (quantification of fluorescent signal as measured by flow cytometry). Improved readthrough upon increased copy number was seen for the 001 and 113 suppressors. Together, the results show that increasing the copy number of the suppressor tRNA modules in reporter constructs
 15 often results in enhanced readthrough activity. Additionally, U6-containing constructs exhibited PTC readthrough activity, although generally not as much as was seen for equivalent suppressor tRNAs expressed in the flanking genomic context.

Example 3

[00201] This example describes the design of a functional arginine aminoacylated
 20 suppressor tRNA that facilitates read-through of a premature termination codon (PTC) in a transcript.

[00202] C57BL/6J mice have a naturally occurring C-to-T mutation in the T-loop (position 51) of Arg-TCT-5-1 that has been shown to affect pre-tRNA processing and function (Ryuta Ishimura et al., *Science*, 2014). Arg_{TCA} suppressor 120 (as described in Example 1 and shown in
 25 **TABLE 8**) contains a T at position 51. A modified suppressor tRNA was generated that included a substitution of C for T at position 51 of Arg_{TCA} suppressor 120 (referred to as Arg_{TCA} suppressor 179 and having a nucleotide sequence of SEQ ID NO: 35). Arg_{TCA} suppressors 120 and 179 were tested by transfection into a Flp-In-293 cell line stably expressing the subject S-PTC Reporter and by co-transfection with a plasmid encoding the subject N-PTC reporter into
 30 Flp-In-3T3 cells. Transfections were done using the Lipofectamine 3000 Transfection Reagent according to the manufacturer's protocol. Results are shown in **FIGURE 4** and **FIGURE 6**. While Arg_{TCA} suppressor 120 was non-functional, Arg_{TCA} suppressor 179, containing only a single substitution, showed PTC readthrough activity.

Example 4

[00203] This Example describes glutamine aminoacylated suppressor tRNAs that facilitate read-through of a premature termination codon (PTC).

[00204] Suppressor tRNAs were generated from endogenous mouse glutamine-tRNAs by converting their normal anticodons to TTA or CTA anticodons (referred to as Gln^{TTA} or Gln^{CTA} suppressor tRNAs, respectively). Suppressor tRNA sequences are shown in **TABLE 9**.

TABLE 9

SEQ ID NO	Suppressor tRNA ID Number	Suppressor tRNA Name	Suppressor tRNA Sequence (anticodon lowercase)
36	002	tRNA-Gln-TTG-1-1-TTA-SUP	GGTCCCATGGTGTAATGGTTAGCAC TCTGGACTttaAATCCAGCGATCCG AGTTCAAATCTCGGTGGGACCT
37	155	tRNA-Gln-CTG-1-1-TTA-SUP	GGTTCCATGGTGTAATGGTTAGCAC TCTGGACTttaAATCCAGCGACCCG AGTTCAAATCTCGGTGGGACCT
38	156	tRNA-Gln-CTG-2-1-TTA-SUP	GGTTCCATGGTGTAATGGTTAGCAC TCTGGACTttaAATCCAGCGATCCG AGTTCAAATCTCGGTGGAACCT
39	157	tRNA-Gln-CTG-3-1-TTA-SUP	GGTTCCATGGTGTAATGGTTAGCAC TCTGGACTttaAATCCAGCGATCCG AGTTCAAATCTCGGTGGGACCT
40	158	tRNA-Gln-CTG-4-1-TTA-SUP	GGTTCCATGGTGTAATGGTGAGCAC TCTGGACTttaAATCCAGCGATCCG AGTTCAAATCTCGGTGGGACCT
41	159	tRNA-Gln-CTG-5-1-TTA-SUP	GGTTCCATGGTGTAATGGCTAGCAC TCTGGACTttaAATCCAGCGATCCG AGTTCAAATCTCGGTGGGATTT
42	160	tRNA-Gln-CTG-6-1-TTA-SUP	GGTTCCATGGTGTAATGGTTAGCAC TCTGGACTttaAATCCAGCCATACA AGTTCAAATCTCAGTGGAACCT

43	161	tRNA-Gln-CTG-7-1-TTA-SUP	GGTTCCTTGGTGTAAGATGAGC ACTCTGGATTttaAATCCAGCG ATCAGAGTTCAAATCTCGGTGG GACCT
44	162	tRNA-Gln-TTG-2-1-TTA-SUP	GGTCCCATGGTGTAATGGTTAG CACTCTGGACTttaAATCCAGC AATCTGAGTTCAAATCTCGGTG GGACCT
45	163	tRNA-Gln-TTG-3-1-TTA-SUP	GGCCCCATGGTGTAATGGTTAG CACTCTGGACTttaAATCCAGC GATCCGAGTTCAAATCTCGGTG GGACCT
46	164	tRNA-Gln-TTG-4-1-TTA-SUP	GGTCTCATGGTGTAATGGTTAG CACACTGGACTttaAGTCCAGC AATCCGAGTTGAGTCTTGGTG AGACCA
47	165	tRNA-Gln-TTG-5-1-TTA-SUP	GGACCCATGGTGTAATGGTTAG CACTCTGGACTttaAATCCAGC AATCCAAGTTCAAATCTCGGTG GGACCT
48	166	tRNA-Gln-TTG-6-1-TTA-SUP	GTTTCCATGGTGTAATGGTTGG CACTCTGGACTttaAATCCAGC AATCCAAGTTCAAGTCTCTGTG GGACCT
178	196	tRNA-Gln-CTG-1-1-CTA-SUP	GGTCCCATGGTGTAATGGTTAG CACTCTGGACTctaAATCCAGC GATCCGAGTTCAAATCTCGGTG GGACCT
179	189	tRNA-Gln-CTG-2-1-CTA-SUP	GGTTCATGGTGTAATGGTTAG CACTCTGGACTctaAATCCAGC GACCCGAGTTCAAATCTCGGTG GGACCT

180	190	tRNA-Gln-CTG-3-1-CTA-SUP	GGTTCCATGGTGTAATGGTTAG CACTCTGGACTctaAATCCAGC GATCCGAGTTCAAATCTCGGTG GAACCT
181	191	tRNA-Gln-CTG-4-1-CTA-SUP	GGTTCCATGGTGTAATGGTTAG CACTCTGGACTctaAATCCAGC GATCCGAGTTCAAATCTCGGTG GGACCT
182	192	tRNA-Gln-CTG-5-1-CTA-SUP	GGTTCCATGGTGTAATGGTGAG CACTCTGGACTctaAATCCAGC GATCCGAGTTCAAATCTCGGTG GGACCT
183	193	tRNA-Gln-CTG-6-1-CTA-SUP	GGTTCCATGGTGTAATGGCTAG CACTCTGGACTctaAATCCAGC GATCCGAGTTCAAATCTCGGTG GGATTT
184	194	tRNA-Gln-CTG-7-1-CTA-SUP	GGTTCCATGGTGTAATGGTTAG CACTCTGGACTctaAATCCAGC CATAACAAGTTCAAATCTCAGTG GAACCT
185	195	tRNA-Gln-TTG-1-1-CTA-SUP	GGTTCCTTGGTGTAAGATGAGC ACTCTGGATTctaAATCCAGCG ATCAGAGTTCAAATCTCGGTGG GACCT
186	197	tRNA-Gln-TTG-2-1-CTA-SUP	GGTCCCATGGTGTAATGGTTAG CACTCTGGACTctaAATCCAGC AATCTGAGTTCAAATCTCGGTG GGACCT
187	198	tRNA-Gln-TTG-3-1-CTA-SUP	GGCCCCATGGTGTAATGGTTAG CACTCTGGACTctaAATCCAGC GATCCGAGTTCAAATCTCGGTG GGACCT

188	199	tRNA-Gln-TTG-4-1-CTA-SUP	GGTCTCATGGTGTAATGGTTAG CACACTGGACTctaAGTCCAGC AATCCGAGTTCGAGTCTTGGTG AGACCA
189	200	tRNA-Gln-TTG-5-1-CTA-SUP	GGACCCATGGTGTAATGGTTAG CACTCTGGACTctaAATCCAGC AATCCAAGTTCAAATCTCGGTG GGACCT
190	201	tRNA-Gln-TTG-6-1-CTA-SUP	GTTTCCATGGTGTAATGGTTGG CACTCTGGACTctaAATCCAGC AATCCAAGTTCAAGTCTCTGTG GGACCT

[00205] In this Example, all mature tRNA sequences were expressed in the context of upstream and downstream genomic flanking sequences (±200 bps) from tRNA-Gln-TTG-1-1 — a highly expressed glutamine-tRNA, i.e., the tRNA sequences were expressed with a 5' flanking sequence of SEQ ID NO: 173 and a 3' flanking sequence of SEQ ID NO: 174. All mature tRNA sequences including upstream and downstream genomic flanking sequences were generated in a pGL4 vector backbone.

[00206] The Gln^{TTA} suppressors were tested for PTC readthrough activity by flow cytometry in two independently derived Flp-In-293 cell lines containing an integrated fluorescent readthrough reporter. Results are shown in **FIGURE 18**. The reporters included three copies of a red fluorescent protein (tdTomato), TEV protease, a linker region containing a PTC, and three copies of a green fluorescent protein (EGFP). A schematic of the reporter construct is shown in **FIGURE 3**. In the absence of any PTC readthrough as a result of a suppressor tRNA, translation will be terminated by the PTC within the linker region, and only tdTomato will be expressed (and therefore only red fluorescence detected). PTC readthrough activity as a result of a suppressor tRNA will allow translation to proceed through the PTC in the linker region, and for both tdTomato and EGFP to be expressed (and therefore both red and green fluorescence detected). Accordingly, readthrough was assessed with flow cytometry by quantifying the percentage of viable cells expressing both the red and green fluorescent reporters above background (double positive %). The linker was derived from a mouse Dmd^{mdx} transcript, and had the following sequence:

CTGCAAAGTTCTTTGAAAGAGCAA_taaAATGGCTTCAACTATCTGAGTGAC (SEQ ID NO: 192).

A corresponding linker with a wild-type Gln codon in place of the PTC was used as a control, and had the following sequence:

CTGCAAAGTTCTTTGAAAGAGCAACAAAATGGCTTCAACTATCTGAGTGAC (SEQ ID NO: 191).

[00207] An additional reporter construct was generated in a pGL4 vector backbone including EGFP with a nuclear localization signal (NLS) and containing a glutamine-to-TAA mutation (Q69X) that abolishes fluorescence in the absence of PTC readthrough. EGFP expression was driven by the CMV early enhancer/chicken β actin (CAG) promoter. The reporter construct is referred to as CAG:NLS-EGFP (Q69X-TAA) and its sequence is as follows:

CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACG
TCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGG
AGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC
TATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGAC
15 TTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCA
CGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTT
TTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGG
CGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGC
TCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCG
20 GCGGGCGGGAGTCGCTGCGCGCTGCCTTCGCCCCGTGCCCCGCTCCGCCGCCGCCTCGCGCCGC
CCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTC
CGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTGCGTGAAAGCCT
TGAGGGGCTCCGGGAGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCG
CCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGG
25 GGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACC
ATGTTTCATGCCTTCTTCTTTTCTTCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCA
TCATTTTGGCAAAGAATTGCGGCCCAACGGTACCGGATCCACCGGCCGCCACCATGGGAAGCCC
AAAGAAGAAGCGTAAGGTAATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATC
CTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCG
30 ATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCCGTGCCCTG
GCCACCCCTCGTGACCACCCTGACCTACGGCGTGTAATGCTTCAGCCGCTACCCCGACCACATG
AAGCAGCACGACTTCTTCAAGTCCGCCATGCCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCT
TCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAA

CCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAG
TACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGA
ACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAA
CACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCCGACAACCACTACCTGAGCACCCAGTCCGCC
5 CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCG
GGATCACTCTCGGCATGGACGAGCTGTACAAGGGAAGCCCCAAGAAAAAGCGGAAGGTGTAA
(SEQ ID NO: 175).

[00208] The activity of the Gln^{TTA} suppressors was assessed by flow cytometry in Neuro-2a cells transiently co-transfected with plasmids encoding a Gln^{TTA} suppressor tRNA and the
10 CAG:NLS-EGFP (Q69X-TAA) reporter. Transfections were done using the Lipofectamine 3000 Transfection Reagent according to the manufacturer's protocol. The results are shown in **FIGURE 19**.

[00209] An additional reporter construct was generated in a pGL4 vector backbone including EGFP with a nuclear localization signal (NLS) and containing a glutamine-to-TAG
15 mutation (Q69X) that abolishes fluorescence in the absence of PTC readthrough. EGFP expression is driven by the CMV early enhancer/chicken β actin (CAG) promoter. The reporter construct is referred to as CAG:NLS-EGFP (Q69X-TAG) and its sequence is as follows:

CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTTGACG
TCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGG
20 AGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC
TATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGAC
TTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTTCGAGGTGAGCCCCA
CGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTT
TTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGG
25 CGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGC
TCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCG
GCGGGCGGGAGTCGCTGCGCGCTGCCTTCGCCCCGTGCCCCGCTCCGCCGCCGCCCTCGCGCCGC
CCGCCCCGGCTCTGACTGACCGCGTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTC
CGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTGCGTGAAAGCCT
30 TGAGGGGCTCCGGGAGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCG
CCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGG
GGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACC
ATGTTTCATGCCTTCTTCTTTTCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCA

TCATTTTGGCAAAGAATTGCGGCCCCAACGGTACCGGATCCACCGGCCGCCACCATGGGAAGCCC
AAAGAAGAAGCGTAAGGTAATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATC
CTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCG
ATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCCGTGCCCTG
5 GCCCACCCTCGTGACCACCCTGACCTACGGCGTGTAGTGCTTCAGCCGCTACCCCGACCACATG
AAGCAGCACGACTTCTTCAAGTCCGCCATGCCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCT
TCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAA
CCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAG
TACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGA
10 ACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAA
CACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCC
CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCG
GGATCACTCTCGGCATGGACGAGCTGTACAAGGGAAGCCCCAAGAAAAAGCGGAAGGTGTAA
(SEQ ID NO: 176).

15 **[00210]** The activity of the GlnCTA suppressors was assessed in Neuro-2a cells transiently co-transfected with plasmids encoding a GlnCTA suppressor tRNA and the CAG:NLS-EGFP (Q69X-TAG) reporter. The results are shown in **FIGURE 20**.

[00211] Together, these results demonstrate that the described suppressor tRNAs can facilitate expression of transcripts containing premature termination codons associated with
20 disorders.

Example 5

[00212] This Example describes readthrough activity of disclosed suppressor tRNAs and small molecule nonsense suppression therapies.

[00213] Disclosed suppressor tRNAs were tested alongside nonsense suppression drugs
25 translarna (ataluren), gentamicin, and G418 (geneticin). PTC readthrough activity was measured in Neuro-2a cells ~48 hours after transfection with an expression construct containing a CAG:NLS-EGFP (R96X-TGA) reporter (as described in Example 1) and either (i) including an ArgTCA suppressor tRNA at the indicated copy number on the same construct, or (ii) treated with ataluren, (iii) treated with gentamicin, or (iv) treated with G418. Transfections were performed
30 using the Lipofectamine 3000 Transfection Reagent according to the manufacturer's protocol. For all experimental conditions, cell culture medium was replaced with fresh medium ~6 hours after transfection and the indicated drugs at the indicated concentrations were added at this point. PTC readthrough activity was measured as the percentage of EGFP positive cells. A reporter

containing wildtype EGFP without a PTC was used as a control. Cell viability in cells receiving the same set of treatments was assessed by flow cytometry following staining with 7-Amino Actinomycin D (7-AAD; Thermo Fisher Scientific #006993-50), a membrane impermeant dye that is generally excluded from viable cells, which was used according to the manufacturer's protocol. The results are shown in **FIGUREs 21-23**. Together, the results demonstrate that the Arg_{TCA} suppressor tRNA #115 (SEQ ID NO: 18) produces far greater readthrough than any of the nonsense suppression drugs. Additionally, the results show that, unlike for any of the nonsense suppression drugs, treatment with the Arg_{TCA} suppressor tRNA is not accompanied by a decrease in cell viability.

Example 6

- [00214] This Example describes aminoacylated suppressor tRNAs that facilitate read-through of a premature termination codon (PTC).
- [00215] Suppressor tRNAs are generated from endogenous mouse tRNAs by converting their normal anticodons to anticodons that recognize premature termination codons (PTCs).
- Suppressor tRNA sequences are shown in **TABLE 10**.

TABLE 10

SEQ ID NO	Suppressor tRNA Name	Suppressor tRNA Sequence
49	pre-tRNA-Leu-CAA->cta--1-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTctaGCTATGGCTTCCTCG CTCTGAGGGTTCTGGTCTCCCCTGGA GGCGTGGGTTCGAATCCCACCTTCTGA CA
50	pre-tRNA-Leu-CAA->cta--2-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTctaGCTTAGCTTCCTGT CTGGGGATTCTGGTCTCCGTATGGAG GCGTGGGTTCGAATCCCACCTTCTGAC A

51	pre-tRNA-Leu-CAA->cta--3-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTctaGGTGACAAGCCTTAC CTACGGGTGTTCTGGTCTCCGAATGG AGGCGTGGGTTCGAATCCCACCTTCTG ACA
52	pre-tRNA-Leu-CAA->cta--4-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTctaGCGTTCGCTTCCTCT ACTGAGGGTTCTGGTCTCCGTGTGGA GGCGTGGGTTCGAATCCCACCTTCTGA CA
53	pre-tRNA-Leu-CAA->tca--1-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTtcaGCTATGGCTTCCTCG CTCTGAGGGTTCTGGTCTCCCCTGGA GGCGTGGGTTCGAATCCCACCTTCTGA CA
54	pre-tRNA-Leu-CAA->tca--2-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTtcaGCTTAGCTTCCTGT CTGGGGATTCTGGTCTCCGTATGGAG GCGTGGGTTCGAATCCCACCTTCTGAC A
55	pre-tRNA-Leu-CAA->tca--3-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTtcaGGTGACAAGCCTTAC CTACGGGTGTTCTGGTCTCCGAATGG AGGCGTGGGTTCGAATCCCACCTTCTG ACA
56	pre-tRNA-Leu-CAA->tca--4-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTtcaGCGTTCGCTTCCTCT ACTGAGGGTTCTGGTCTCCGTGTGGA GGCGTGGGTTCGAATCCCACCTTCTGA CA

57	pre-tRNA-Leu-CAA->tta--1-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTttaGCTATGGCTTCCTCG CTCTGAGGGTTCTGGTCTCCCCTGGA GGCGTGGGTTCGAATCCCACCTTCTGA CA
58	pre-tRNA-Leu-CAA->tta--2-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTttaGCTTAGCTTCCTGT CTGGGGATTCTGGTCTCCGTATGGAG GCGTGGGTTCGAATCCCACCTTCTGAC A
59	pre-tRNA-Leu-CAA->tta--3-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTttaGGTGACAAGCCTTAC CTACGGGTGTTCTGGTCTCCGAATGG AGGCGTGGGTTCGAATCCCACCTTCTG ACA
60	pre-tRNA-Leu-CAA->tta--4-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTttaGCGTTCGCTTCCTCT ACTGAGGGTTCTGGTCTCCGTGTGGA GGCGTGGGTTCGAATCCCACCTTCTGA CA
61	pre-tRNA-Tyr-GTA->cta--1-1	CCTTCGATAGCTCAGTTGGTAGAGCG GAGGACTctaGAGTTACTAGAATAGT GATCCTTAGGTCGCTGGTTCGAATCC GGCTCGAAGGA
62	pre-tRNA-Tyr-GTA->cta--2-1	CCTTCGATAGCTCAGTTGGTAGAGCG GAGGACTctaGTCAGTACAATATGGT AATCCTTAGGTCGCTGGTTCGATTCC GGCTCGAAGGA
63	pre-tRNA-Tyr-GTA->cta--3-1	CCTTCGATAGCTCAGCTGGTAGAGCG GAGGACTctaGGCTTGTGGCTGTGGA CATCCTTAGGTCGCTGGTTCGATTCC GGCTCGAAGGA

64	pre-tRNA-Tyr-GTA->cta--4-1	CCTTCGATAGCTCAGCTGGTAGAGCG GAGGACTctaGCTAACTCCCCGTTAG AAGACATCCTTAGGTCGCTGGTTCGA CTCCGGCTCGAAGGA
65	pre-tRNA-Tyr-GTA->cta--5-1	CTTTCGATAGTTCAGTTGGTAGAGCG GAGGACTctaGAGTATTAACGTTGGT GATCCTTAGGTCGCTGGTTCGAGTCC GGCTCGAAGGA
66	pre-tRNA-Tyr-GTA->tta--1-1	CCTTCGATAGCTCAGTTGGTAGAGCG GAGGACTttaGAGTTACTAGAATAGT GATCCTTAGGTCGCTGGTTCGAATCC GGCTCGAAGGA
67	pre-tRNA-Tyr-GTA->tta--2-1	CCTTCGATAGCTCAGTTGGTAGAGCG GAGGACTttaGTCAGTACAATATGGT AATCCTTAGGTCGCTGGTTCGATTCC GGCTCGAAGGA
68	pre-tRNA-Tyr-GTA->tta--3-1	CCTTCGATAGCTCAGCTGGTAGAGCG GAGGACTttaGGCTTGTGGCTGTGGA CATCCTTAGGTCGCTGGTTCGATTCC GGCTCGAAGGA
69	pre-tRNA-Tyr-GTA->tta--4-1	CCTTCGATAGCTCAGCTGGTAGAGCG GAGGACTttaGCTAACTCCCCGTTAG AAGACATCCTTAGGTCGCTGGTTCGA CTCCGGCTCGAAGGA
70	pre-tRNA-Tyr-GTA->tta--5-1	CTTTCGATAGTTCAGTTGGTAGAGCG GAGGACTttaGAGTATTAACGTTGGT GATCCTTAGGTCGCTGGTTCGAGTCC GGCTCGAAGGA
71	tRNA-Cys-GCA->tca--1-1	GGGGGTATAGCTCAGTGGTAGAGCAT TTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
72	tRNA-Cys-GCA->tca--10-1	GGGGGTATAGCTCAGGGGTAGAGCAT TTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGATGCCCCCT

73	tRNA-Cys-GCA->tca--11-1	GGGGGTATAGCTCAGGGGTAGAGTAT TTGGCTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
74	tRNA-Cys-GCA->tca--12-1	GGGGGTATAGCTCAGGGGTAGAGCAT TTGACTtcaGATCAAGAGGTCCTTGG TTCAAATCCAGGTGTCCCCCT
75	tRNA-Cys-GCA->tca--13-1	GGGGGTATAGCTCAGAGGTAGAGCAT TTGACTtcaGATCAAGAGATCTCTGG TTCAAATCCAGGTGCCCCCT
76	tRNA-Cys-GCA->tca--14-1	GGGGGTATAGCTCAGGGGTAGAGCAT TTGACTtcaGATCAAGAGGTCCCTAG TTCAAATCCAGGTGCCCCCT
77	tRNA-Cys-GCA->tca--15-1	GGTGGTATAGCTCAGGGGTAGAGCAT TTGACTtcaGATCAAGAGATCCCTGG TTCGAATCCAGGTGCCCCCT
78	tRNA-Cys-GCA->tca--16-1	GGGGGTATAACTCAGGGGTAGAGCAT TTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
79	tRNA-Cys-GCA->tca--17-1	TGGGGTATAGCTCAGGGGTAGAGCAT TTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
80	tRNA-Cys-GCA->tca--18-1	GGGGGTATAGCTCAGAGGAAGAGCAT TTGACTtcaGATCAAGAGGTCCCTGA TTCAAATCCAGGTGCCCCCT
81	tRNA-Cys-GCA->tca--19-1	GGGGGTAAAGCTCAGGGGTAGAGCAT TTGACTtcaGATTAAGAGGTCCCTGG TTCAAATCCAGGTACCCCCCT
82	tRNA-Cys-GCA->tca--2-1	GGGGGTATAGCTCAGTGGTAGAGCAT TTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCGGGTGCCCCCT
83	tRNA-Cys-GCA->tca--21-1	GGGGTTATAGCTCAGGTGTAGAGCAT TTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT

84	tRNA-Cys-GCA->tca--24-1	GGGGGTATAGCTCAGGGGTAGAGCAT TTGACTtcaGATCACGAGGTCCCTGG TTCAAATCGAGGTGCCCCCT
85	tRNA-Cys-GCA->tca--25-1	GGGGGTATAGCTCAGGGGTGGAGCAT TTGACTtcaGATCAAGGGGTCCCTGT TTCAAATCCAGGTGCCCCCT
86	tRNA-Cys-GCA->tca--3-1	GGGGGTATAGCTCAGTGGTAGAGCAT TTGACTtcaGATCAAGAGGTCCCCGG TTCAAATCCGGGTGCCCCCT
87	tRNA-Cys-GCA->tca--4-1	GGGGGTATAGCTCAGGGGTAGAGCAT TTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
88	tRNA-Cys-GCA->tca--5-1	GGGGGTATAGCTCAGGGGTAGAGCAT TTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCGGGTGCCCCCT
89	tRNA-Cys-GCA->tca--6-1	GGGGGTATAGCTCAGGGGTAGAGCAT TTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTACCCCCCT
90	tRNA-Cys-GCA->tca--7-1	GGGGGTATAGCTCAGGGGTAGAGCAT TTGACTtcaGATCAAGAGGTCCCCGG TTCAAATCCGGGTGCCCCCT
91	tRNA-Cys-GCA->tca--8-1	GGGGGCATAGCTCAGGGGTAGAGCAT TTGACTtcaGATCAAGAGGTCCCCGG TTCAAATCCGGGTGCTCCCT
92	tRNA-Cys-GCA->tca--9-1	GGGGGTATAGCTCAGGGGTAGAGCAT TTGACTtcaGATTAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
93	tRNA-Glu-CTC->cta--1-1	TCCCTGGTGGTCTAGTGGTTAGGATT CGGCGCTctaACCGCCGCGGCCCGGG TTCGATTCCCGGTCAGGGAA
94	tRNA-Glu-CTC->cta--2-1	TCCCTGGTGGTCTAGTGGTTAGGATT TGGCGCTctaACCGCCGCGGCCTGGG TTCGATTCCCGGTCAGGGAA

95	tRNA-Glu-CTC->cta--5-1	TCCCTGGTGGTCTAGTGGTTAGGCTT TGGTGCTctaACCTCCATGGCCCAGG TTTGATTCCTGGTCAGGGAA
96	tRNA-Glu-CTC->tta--1-1	TCCCTGGTGGTCTAGTGGTTAGGATT CGGCGCTttaACCGCCGCGGCCCGGG TTCGATTCCCGGTCAGGGAA
97	tRNA-Glu-CTC->tta--2-1	TCCCTGGTGGTCTAGTGGTTAGGATT TGGCGCTttaACCGCCGCGGCCTGGG TTCGATTCCCGGTCAGGGAA
98	tRNA-Glu-CTC->tta--5-1	TCCCTGGTGGTCTAGTGGTTAGGCTT TGGTGCTttaACCTCCATGGCCCAGG TTTGATTCCTGGTCAGGGAA
99	tRNA-Glu-TTC->cta--1-1	TCCCACATGGTCTAGCGGTTAGGATT CCTGGTTctaACCCAGGCGGCCCGGG TTCGACTCCCGGTGTGGGAA
100	tRNA-Glu-TTC->cta--2-1	TCCCATATGGTCTAGCGGTTAGGATT CCTGGTTctaACCCAGGCGGCCCGGG TTCGACTCCCGGTATGGGAA
101	tRNA-Glu-TTC->cta--3-1	TCCCTGGTGGTCTAGTGGCTAGGATT CGGCGCTctaACCGCCGCGGCCCGGG TTCGATTCCCGGTCAGGGAA
102	tRNA-Glu-TTC->tta--1-1	TCCCACATGGTCTAGCGGTTAGGATT CCTGGTTttaACCCAGGCGGCCCGGG TTCGACTCCCGGTGTGGGAA
103	tRNA-Glu-TTC->tta--2-1	TCCCATATGGTCTAGCGGTTAGGATT CCTGGTTttaACCCAGGCGGCCCGGG TTCGACTCCCGGTATGGGAA
104	tRNA-Glu-TTC->tta--3-1	TCCCTGGTGGTCTAGTGGCTAGGATT CGGCGCTttaACCGCCGCGGCCCGGG TTCGATTCCCGGTCAGGGAA
105	tRNA-Gly-ACC->tca--1-1	GTTTCCGTAGTGTAGTGGTTAGCGCG TTCGCCTtcaAAAGCGAAAGGTCCCC GGTTCGAAACCGGGCGGAAACA

106	tRNA-Gly-CCC->tca--1-1	GCGCCGCTGGTGTAGTGGTATCATGC AAGATTtcaATTCTTGCGACCCGGGT TCGATTCCCGGGCGGCGCA
107	tRNA-Gly-CCC->tca--2-1	GCATTGGTAGTTCAATGGTAGAATTC TCGCCTtcaACGCGGGTGACCCGGGT TCGATTCCCGGCCAATGCA
108	tRNA-Gly-CCC->tca--3-1	GCATTGGTGGTTCAATGGTAGAATTC TCGCCTtcaACGCGGGTGACCCGGGT TCGATTCCCGGCCAATGCA
109	tRNA-Gly-CCC->tca--4-1	GCATTGGTGGTTCAATGGTAGAATTC TCGCCTtcaACTCGGGTGACCCGGGT TCGATTCCCGGCCAATGCA
110	tRNA-Gly-GCC->tca--1-1	GCATGGGTGGTTCAGTGGTAGAATTC TCGCCTtcaACGCGGGAGGCCCGGGT TCGATTCCCGGCCCATGCA
111	tRNA-Gly-GCC->tca--2-1	GCATTGGTGGTTCAGTGGTAGAATTC TCGCCTtcaACGCGGGAGGCCCGGGT TCGATTCCCGGCCAATGCA
112	tRNA-Gly-GCC->tca--3-1	GCATTGGTGGTTCAGTGGTAGAATTC TCGCCTtcaACGCGGGAGGCCCGGGT TTGATTCCCGGCCAATGCA
113	tRNA-Gly-GCC->tca--4-1	GCATTGGTGGTTCAGTGGTAGAATTC TCGCCTtcaACGCGGGAGGCCCGGGT TCGGTTCCCGGCCAATGCA
114	tRNA-Gly-TCC->tca--1-1	GCGTTGGTGGTATAGTGGTGAGCATA GCTGCCTtcaAAGCAGTTGACCCGGG TTCGATTCCCGGCCAACGCA
115	tRNA-Leu-AAG->cta--1-1	GGTAGCGTGGCCGAGCGGTCTAAGGC GCTGGATTctaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCT GCCA

116	tRNA-Leu-AAG->cta--2-1	GGTAGTGTGGCCGAGCGGTCTAAGGC GCTGGATTctaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCT GCCA
117	tRNA-Leu-AAG->cta--3-1	GGTAGTGTGGCCGAGCGGTCTAAGGC GCTGGATTctaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCACT GCCA
118	tRNA-Leu-AAG->tca--1-1	GGTAGCGTGGCCGAGCGGTCTAAGGC GCTGGATTtcaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCT GCCA
119	tRNA-Leu-AAG->tca--2-1	GGTAGTGTGGCCGAGCGGTCTAAGGC GCTGGATTtcaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCT GCCA
120	tRNA-Leu-AAG->tca--3-1	GGTAGTGTGGCCGAGCGGTCTAAGGC GCTGGATTtcaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCACT GCCA
121	tRNA-Leu-AAG->tta--1-1	GGTAGCGTGGCCGAGCGGTCTAAGGC GCTGGATTttaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCT GCCA
122	tRNA-Leu-AAG->tta--2-1	GGTAGTGTGGCCGAGCGGTCTAAGGC GCTGGATTttaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCT GCCA
123	tRNA-Leu-AAG->tta--3-1	GGTAGTGTGGCCGAGCGGTCTAAGGC GCTGGATTttaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCACT GCCA

124	tRNA-Leu-CAA->cta--1-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTctaGTTCTGGTCTCCCCT GGAGGCGTGGGTTCGAATCCCCTTC TGACA
125	tRNA-Leu-CAA->cta--2-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTctaGTTCTGGTCTCCGTA TGGAGGCGTGGGTTCGAATCCCCTT CTGACA
126	tRNA-Leu-CAA->cta--3-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTctaGTTCTGGTCTCCGAA TGGAGGCGTGGGTTCGAATCCCCTT CTGACA
127	tRNA-Leu-CAA->cta--4-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTctaGTTCTGGTCTCCGTG TGGAGGCGTGGGTTCGAATCCCCTT CTGACA
128	tRNA-Leu-CAA->tca--1-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTtcaGTTCTGGTCTCCCCT GGAGGCGTGGGTTCGAATCCCCTTC TGACA
129	tRNA-Leu-CAA->tca--2-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTtcaGTTCTGGTCTCCGTA TGGAGGCGTGGGTTCGAATCCCCTT CTGACA
130	tRNA-Leu-CAA->tca--3-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTtcaGTTCTGGTCTCCGAA TGGAGGCGTGGGTTCGAATCCCCTT CTGACA
131	tRNA-Leu-CAA->tca--4-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTtcaGTTCTGGTCTCCGTG TGGAGGCGTGGGTTCGAATCCCCTT CTGACA

132	tRNA-Leu-CAA->tta--1-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTttaGTTCTGGTCTCCCCT GGAGGCGTGGGTTCGAATCCCCTTC TGACA
133	tRNA-Leu-CAA->tta--2-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTttaGTTCTGGTCTCCGTA TGGAGGCGTGGGTTCGAATCCCCTT CTGACA
134	tRNA-Leu-CAA->tta--3-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTttaGTTCTGGTCTCCGAA TGGAGGCGTGGGTTCGAATCCCCTT CTGACA
135	tRNA-Leu-CAA->tta--4-1	GTCAGGATGGCCGAGTGGTCTAAGGC GCCAGACTttaGTTCTGGTCTCCGTG TGGAGGCGTGGGTTCGAATCCCCTT CTGACA
136	tRNA-Leu-CAG->cta--1-1	GTCAGGATGGCCGAGCGGTCTAAGGC GCTGCGTTctaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCCTCC TGACA
137	tRNA-Leu-CAG->cta--2-1	GTCAGGATGGCCGAGCGGTCTAAGGC GCTGCGTTctaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCCTTC TGACA
138	tRNA-Leu-CAG->cta--3-1	GTCAGGATGGCCGAGTGGTCTAAGGA GCTGTGTTctaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCCTCC TGACA
139	tRNA-Leu-CAG->cta--4-1	GTCAGGATGGCCGAGCAGTCTAAGGC ACTGCGTTctaGTCGCAGTCTCCCCT GGAGGCGTGGATTTCGAATCCCCTCC TGACA

140	tRNA-Leu-CAG->tca--1-1	GTCAGGATGGCCGAGCGGTCTAAGGC GCTGCGTTtcaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCCTCC TGACA
141	tRNA-Leu-CAG->tca--2-1	GTCAGGATGGCCGAGCGGTCTAAGGC GCTGCGTTtcaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCCTTC TGACA
142	tRNA-Leu-CAG->tca--3-1	GTCAGGATGGCCGAGTGGTCTAAGGA GCTGTGTTtcaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCCTCC TGACA
143	tRNA-Leu-CAG->tca--4-1	GTCAGGATGGCCGAGCAGTCTAAGGC ACTGCGTTtcaGTCGCAGTCTCCCCT GGAGGCGTGGATTTCGAATCCCCTCC TGACA
144	tRNA-Leu-CAG->tta--1-1	GTCAGGATGGCCGAGCGGTCTAAGGC GCTGCGTTttaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCCTCC TGACA
145	tRNA-Leu-CAG->tta--2-1	GTCAGGATGGCCGAGCGGTCTAAGGC GCTGCGTTttaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCCTTC TGACA
146	tRNA-Leu-CAG->tta--3-1	GTCAGGATGGCCGAGTGGTCTAAGGA GCTGTGTTttaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCCTCC TGACA
147	tRNA-Leu-CAG->tta--4-1	GTCAGGATGGCCGAGCAGTCTAAGGC ACTGCGTTttaGTCGCAGTCTCCCCT GGAGGCGTGGATTTCGAATCCCCTCC TGACA

148	tRNA-Leu-TAA->cta--1-1	ACCAGAATGGCCGAGTGGTTAAGGCG TTGGACTctaGATCCAATGGATTTAT ATCCGCGTGGGTTCGAACCCCACTTC TGGTA
149	tRNA-Leu-TAA->cta--2-1	ACCAGGATGGCCGAGTGGTTAAGGCG TTGGACTctaGATCCAATGGACATAT GTCTGCGTGGGTTCGAACCCCACTCC TGGTA
150	tRNA-Leu-TAA->cta--3-1	ACTGGGATGGCTGAGTGGTTAAGGCG TTGGACTctaGATCCAATGGGCGGTT GCCTGCGTGGGTTCGAACCCCACTCC CAGTA
151	tRNA-Leu-TAA->cta--4-1	GATGGGATGGCTGAGAGGTTAAGGCT TTGGACTctaGATCCAATGGGCAGAT GCCTGCGTGGGTTTGAACCCCACTCC CAATA
152	tRNA-Leu-TAA->tca--1-1	ACCAGAATGGCCGAGTGGTTAAGGCG TTGGACTtcaGATCCAATGGATTTAT ATCCGCGTGGGTTCGAACCCCACTTC TGGTA
153	tRNA-Leu-TAA->tca--2-1	ACCAGGATGGCCGAGTGGTTAAGGCG TTGGACTtcaGATCCAATGGACATAT GTCTGCGTGGGTTCGAACCCCACTCC TGGTA
154	tRNA-Leu-TAA->tca--3-1	ACTGGGATGGCTGAGTGGTTAAGGCG TTGGACTtcaGATCCAATGGGCGGTT GCCTGCGTGGGTTCGAACCCCACTCC CAGTA
155	tRNA-Leu-TAA->tca--4-1	GATGGGATGGCTGAGAGGTTAAGGCT TTGGACTtcaGATCCAATGGGCAGAT GCCTGCGTGGGTTTGAACCCCACTCC CAATA

156	tRNA-Leu-TAA->tta--1-1	ACCAGAATGGCCGAGTGGTTAAGGCG TTGGACTttaGATCCAATGGATTTAT ATCCGCGTGGGTTCGAACCCCACTTC TGGTA
157	tRNA-Leu-TAA->tta--2-1	ACCAGGATGGCCGAGTGGTTAAGGCG TTGGACTttaGATCCAATGGACATAT GTCTGCGTGGGTTCGAACCCCACTCC TGGTA
158	tRNA-Leu-TAA->tta--3-1	ACTGGGATGGCTGAGTGGTTAAGGCG TTGGACTttaGATCCAATGGGCGGTT GCCTGCGTGGGTTCGAACCCCACTCC CAGTA
159	tRNA-Leu-TAA->tta--4-1	GATGGGATGGCTGAGAGGTTAAGGCT TTGGACTttaGATCCAATGGGCAGAT GCCTGCGTGGGTTTGAACCCCACTCC CAATA
160	tRNA-Leu-TAG->cta--1-1	GGTAGCGTGGCCGAGCGGTCTAAGGC GCTGGATTctaGCTCCAGTCTCTTCG GAGGCGTGGGTTCGAATCCCACCGCT GCCA
161	tRNA-Leu-TAG->cta--2-1	GGTAGTGTGGCCGAGCGGTCTAAGGC GCTGGATTctaGCTCCAGTCTCTTCG GAGGCGTGGGTTCGAATCCCACCACT GCCA
162	tRNA-Leu-TAG->cta--3-1	GGTAGCGTGGCCGAGTGGTCTAAGGC GCTGGATTctaGCTCCAGTCATTTTCG ATGGCGTGGGTTCGAATCCCACCGCT GCCA
163	tRNA-Leu-TAG->tca--1-1	GGTAGCGTGGCCGAGCGGTCTAAGGC GCTGGATTtcaGCTCCAGTCTCTTCG GAGGCGTGGGTTCGAATCCCACCGCT GCCA

164	tRNA-Leu-TAG->tca--2-1	GGTAGTGTGGCCGAGCGGTCTAAGGC GCTGGATTtcaGCTCCAGTCTCTTCG GAGGCGTGGGTTCGAATCCCACCACT GCCA
165	tRNA-Leu-TAG->tca--3-1	GGTAGCGTGGCCGAGTGGTCTAAGGC GCTGGATTtcaGCTCCAGTCATTTTCG ATGGCGTGGGTTCGAATCCCACCGCT GCCA
166	tRNA-Leu-TAG->tta--1-1	GGTAGCGTGGCCGAGCGGTCTAAGGC GCTGGATTttaGCTCCAGTCTCTTCG GAGGCGTGGGTTCGAATCCCACCGCT GCCA
167	tRNA-Leu-TAG->tta--2-1	GGTAGTGTGGCCGAGCGGTCTAAGGC GCTGGATTttaGCTCCAGTCTCTTCG GAGGCGTGGGTTCGAATCCCACCACT GCCA
168	tRNA-Leu-TAG->tta--3-1	GGTAGCGTGGCCGAGTGGTCTAAGGC GCTGGATTttaGCTCCAGTCATTTTCG ATGGCGTGGGTTCGAATCCCACCGCT GCCA
169	tRNA-Lys-CTT->cta--1-1	GCCCAGCTAGCTCAGTTGGTAGAGCG TGGGACTctaAATCCTAGGGTCGTGG GTTTGAACCCACGTTGGGCG
170	tRNA-Lys-CTT->cta--12-1	GCCCAGCTAGCTCAGTCTGTAGAGCA TGAGACTctaAGTCTCAGGGTCATGG GTTGGAGCCCCATGTTGTGCA
171	tRNA-Lys-CTT->cta--13-1	GCCTAGCTAGTTCAGTCGGTAGAGCA TGAGACTctaAATCTCAGGTTTCATGA GTTTGAGCCCCATGTTGGTTTGGCA
172	tRNA-Lys-CTT->cta--14-1	CCCCGGCTAGCTCAGTCAGTAGAGCT TGAGAATctaAATCTCAGGGTCGTGG GTTGGAGCCCCACGTTGGGCG

196	tRNA-Lys-CTT->cta--2-1	GCCCGGCTAGCTCAGTCGGTAGAGCA TGGGACTctaAATCCCAGGGTCGTGG GTTCGAGCCCCACGTTGGGCG
197	tRNA-Lys-CTT->cta--3-1	GCCCGGCTAGCTCAGTCGGTAGAGCA TGAGACTctaAATCTCAGGGTCGTGG GTTCGAGCCCCACGTTGGGCG
198	tRNA-Lys-CTT->cta--4-1	GCCCAGCTAGCTCAGTCTGTAGAGCA TGAGACTctaAATCTCAGGGTCGTGA GTTCGAGCCCCACGTTGGGTG
199	tRNA-Lys-CTT->cta--5-1	GCCCAGATAGCTCAGTGGGTAGAGCA TGAGACTctaAATCTCAGGGTCATGG GTTTCATGCCCCATGTTGGGTA
200	tRNA-Lys-CTT->cta--6-1	GTCTTGCTGGCTCAGTCGGTACAGCA TGGGACTctaAATCCCAGGGTCGTGG GTTCGAGCTCCACGTTGGGTA
201	tRNA-Lys-CTT->cta--7-1	GCCTGGCTAGCTCAGTCCATAGAGCA TGGGACTctaAATCCCAGGGTCATGG GTTCGAGCCCCATATTAGGCA
202	tRNA-Lys-CTT->cta--8-1	GCCCAGCTAGCTTAGTTGGTAGAGCA TGAGACTctaAATCTCAGAGTCATGG GTTTCAGGCCTCATGTTTGGCA
203	tRNA-Lys-CTT->cta--9-1	AACCTGGCTAGGTCAGTTGGTAGATC ATGAGACTctaAATCTCAGGGTCATG GGTTCAAGCCCCATGTTGGTTT
204	tRNA-Lys-CTT->tta--1-1	GCCCAGCTAGCTCAGTTGGTAGAGCG TGGGACTttaAATCCTAGGGTCGTGG GTTCGAACCCCCACGTTGGGCG
205	tRNA-Lys-CTT->tta--12-1	GCCCAGCTAGCTCAGTCTGTAGAGCA TGAGACTttaAGTCTCAGGGTCATGG GTTGGAGCCCCATGTTGTGCA
206	tRNA-Lys-CTT->tta--13-1	GCCTAGCTAGTTCAGTCGGTAGAGCA TGAGACTttaAATCTCAGGTTTCATGA GTTTGAGCCCCATGTTGGTTTGGCA

207	tRNA-Lys-CTT->tta--14-1	CCCCGGCTAGCTCAGTCAGTAGAGCT TGAGAATttaAATCTCAGGGTCGTGG GTTGGAGCCCCACGTTGGGCG
208	tRNA-Lys-CTT->tta--2-1	GCCCCGGCTAGCTCAGTCGGTAGAGCA TGGGACTttaAATCCCAGGGTCGTGG GTTTCGAGCCCCACGTTGGGCG
209	tRNA-Lys-CTT->tta--3-1	GCCCCGGCTAGCTCAGTCGGTAGAGCA TGAGACTttaAATCTCAGGGTCGTGG GTTTCGAGCCCCACGTTGGGCG
210	tRNA-Lys-CTT->tta--4-1	GCCCAGCTAGCTCAGTCTGTAGAGCA TGAGACTttaAATCTCAGGGTCGTGA GTTTCGAGCCCCACGTTGGGTG
211	tRNA-Lys-CTT->tta--5-1	GCCCAGATAGCTCAGTGGGTAGAGCA TGAGACTttaAATCTCAGGGTCATGG GTTTCATGCCCCATGTTGGGTA
212	tRNA-Lys-CTT->tta--6-1	GTCCTGCTGGCTCAGTCGGTACAGCA TGGGACTttaAATCCCAGGGTCGTGG GTTTCGAGCTCCACGTTGGGTA
213	tRNA-Lys-CTT->tta--7-1	GCCTGGCTAGCTCAGTCCATAGAGCA TGGGACTttaAATCCCAGGGTCATGG GTTTCGAGCCCCATATTAGGCA
214	tRNA-Lys-CTT->tta--8-1	GCCCAGCTAGCTTAGTTGGTAGAGCA TGAGACTttaAATCTCAGAGTCATGG GTTTCAGGCCTCATGTTTGGCA
215	tRNA-Lys-CTT->tta--9-1	AACCTGGCTAGGTCAGTTGGTAGATC ATGAGACTttaAATCTCAGGGTCATG GGTTCAAGCCCCATGTTGGTTT
216	tRNA-Lys-TTT->cta--1-1	GCCCCGATAGCTCAGTCGGTAGAGCA TCAGACTctaAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTTCGGGCG
217	tRNA-Lys-TTT->cta--2-1	GCCTGGATAGCTCAGTCGGTAGAGCA TCAGACTctaAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTTCAGGCG

218	tRNA-Lys-TTT->cta--3-1	GCCTGGATAGCTCAATTGGTAGAGCA TCAGACTctaAATCTGAGGGTTCAGG GTTCAAGTCCCTGTTTCAGGCG
219	tRNA-Lys-TTT->cta--4-1	GCCCAGCCAGCTCAGTAGGTAGAGTA TGAGACTctaAATCTCAGGGTGGTGG GTTTCGAGCCCCATGTTGGGGG
220	tRNA-Lys-TTT->cta--5-1	TGTGGTGTAGCTCAGTCGGTAGAGCA TCAGACTctaAATCTGAGGGTCCAGG GTTTCAGGTCCCTGTTTCGGGTGCCAAA A
221	tRNA-Lys-TTT->tta--1-1	GCCCGGATAGCTCAGTCGGTAGAGCA TCAGACTttaAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTTCGGGCG
222	tRNA-Lys-TTT->tta--2-1	GCCTGGATAGCTCAGTCGGTAGAGCA TCAGACTttaAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTTCAGGCG
223	tRNA-Lys-TTT->tta--3-1	GCCTGGATAGCTCAATTGGTAGAGCA TCAGACTttaAATCTGAGGGTTCAGG GTTCAAGTCCCTGTTTCAGGCG
224	tRNA-Lys-TTT->tta--4-1	GCCCAGCCAGCTCAGTAGGTAGAGTA TGAGACTttaAATCTCAGGGTGGTGG GTTTCGAGCCCCATGTTGGGGG
225	tRNA-Lys-TTT->tta--5-1	TGTGGTGTAGCTCAGTCGGTAGAGCA TCAGACTttaAATCTGAGGGTCCAGG GTTTCAGGTCCCTGTTTCGGGTGCCAAA A
226	tRNA-Ser-AGA->cta--1-1	GTAGTCGTGGCCGAGTGGTTAAGGCG ATGGACTctaAATCCATTGGGGTTTC CCCGCGCAGGTTCGAATCCTGCCGAC TACG
227	tRNA-Ser-AGA->cta--2-1	GTAGTCGTGGCCGAGTGGTTAAGGCG ATGGACTctaAATCCATTGGGGTCTC CCCGCGCAGGTTCGAATCCTGCCGAC TACG

228	tRNA-Ser-AGA->cta--3-1	GTAGTCGTGGCCAAGTGAGTAAGGCA ATGGACTctaAATCCATTGGGGTCTC CCAGCACAGGTTCAAATCCTGCTGAC TATG
229	tRNA-Ser-AGA->tca--1-1	GTAGTCGTGGCCGAGTGGTTAAGGCG ATGGACTtcaAATCCATTGGGGTTTC CCCGCGCAGGTTCGAATCCTGCCGAC TACG
230	tRNA-Ser-AGA->tca--2-1	GTAGTCGTGGCCGAGTGGTTAAGGCG ATGGACTtcaAATCCATTGGGGTCTC CCCGCGCAGGTTCGAATCCTGCCGAC TACG
231	tRNA-Ser-AGA->tca--3-1	GTAGTCGTGGCCAAGTGAGTAAGGCA ATGGACTtcaAATCCATTGGGGTCTC CCAGCACAGGTTCAAATCCTGCTGAC TATG
232	tRNA-Ser-AGA->tta--1-1	GTAGTCGTGGCCGAGTGGTTAAGGCG ATGGACTttaAATCCATTGGGGTTTC CCCGCGCAGGTTCGAATCCTGCCGAC TACG
233	tRNA-Ser-AGA->tta--2-1	GTAGTCGTGGCCGAGTGGTTAAGGCG ATGGACTttaAATCCATTGGGGTCTC CCCGCGCAGGTTCGAATCCTGCCGAC TACG
234	tRNA-Ser-AGA->tta--3-1	GTAGTCGTGGCCAAGTGAGTAAGGCA ATGGACTttaAATCCATTGGGGTCTC CCAGCACAGGTTCAAATCCTGCTGAC TATG
235	tRNA-Ser-CGA->cta--1-1	GCTGTGATGGCCGAGTGGTTAAGGCG TTGGACTctaAATCCAATGGGGTCTC CCCGCGCAGGTTCGAATCCTGCTCAC AGCG

236	tRNA-Ser-CGA->cta--2-1	GTCACGGTGGCCGAGTGGTTAAGGCG TTGGACTctaAATCCAATGGGGTTTC CCCGCACAGGTTCGAATCCTGTTCGT GACG
237	tRNA-Ser-CGA->cta--3-1	GCTGTGATGGCCGAGTGGTTAAGGCG TTGGACTctaAATCCAATGGGGTTCTT CCCGCGCAGGTTCAAATCCTGCTCAC AGCG
238	tRNA-Ser-CGA->tca--1-1	GCTGTGATGGCCGAGTGGTTAAGGCG TTGGACTtcaAATCCAATGGGGTCTC CCCGCGCAGGTTCGAATCCTGCTCAC AGCG
239	tRNA-Ser-CGA->tca--2-1	GTCACGGTGGCCGAGTGGTTAAGGCG TTGGACTtcaAATCCAATGGGGTTTC CCCGCACAGGTTCGAATCCTGTTCGT GACG
240	tRNA-Ser-CGA->tca--3-1	GCTGTGATGGCCGAGTGGTTAAGGCG TTGGACTtcaAATCCAATGGGGTTCTT CCCGCGCAGGTTCAAATCCTGCTCAC AGCG
241	tRNA-Ser-CGA->tta--1-1	GCTGTGATGGCCGAGTGGTTAAGGCG TTGGACTttaAATCCAATGGGGTCTC CCCGCGCAGGTTCGAATCCTGCTCAC AGCG
242	tRNA-Ser-CGA->tta--2-1	GTCACGGTGGCCGAGTGGTTAAGGCG TTGGACTttaAATCCAATGGGGTTTC CCCGCACAGGTTCGAATCCTGTTCGT GACG
243	tRNA-Ser-CGA->tta--3-1	GCTGTGATGGCCGAGTGGTTAAGGCG TTGGACTttaAATCCAATGGGGTTCTT CCCGCGCAGGTTCAAATCCTGCTCAC AGCG

244	tRNA-Ser-GCT->cta--1-1	GACGAGGTGGCCGAGTGGTTAAGGCG ATGGACTctaAATCCATTGTGCTCTG CACGCGTGGGTTCGAATCCCACCTTC GTCG
245	tRNA-Ser-GCT->cta--2-1	GACGAGGTGGCCGAGTGGTTAAGGCG ATGGACTctaAATCCATTGTGCTCTG CACGCATGGGTTCGAATCCCATCCTC GTCG
246	tRNA-Ser-GCT->cta--3-1	GACGAGGTGGCCGAGTGGTTAAGGCG ATGGACTctaAATCCATTGTGCTTTG CACGCGTGGGTTCGAATCCCATCCTC GTCG
247	tRNA-Ser-GCT->cta--4-1	GACGAGGTGGCCGAGTGGTTAAGGCG ATGGACTctaAATCCATTGTGCTCTG CACGCGTGGGTTCGAATCCCATCCTC GTCG
248	tRNA-Ser-GCT->cta--5-1	GATGAGGTGGCCGAGTGGTTAAGGCG ATGGACTctaAATCCATTGTGCTCTG CACGCATGGGTTCGAATCCCATCCTC ATCG
249	tRNA-Ser-GCT->tca--1-1	GACGAGGTGGCCGAGTGGTTAAGGCG ATGGACTtcaAATCCATTGTGCTCTG CACGCGTGGGTTCGAATCCCACCTTC GTCG
250	tRNA-Ser-GCT->tca--2-1	GACGAGGTGGCCGAGTGGTTAAGGCG ATGGACTtcaAATCCATTGTGCTCTG CACGCATGGGTTCGAATCCCATCCTC GTCG
251	tRNA-Ser-GCT->tca--3-1	GACGAGGTGGCCGAGTGGTTAAGGCG ATGGACTtcaAATCCATTGTGCTTTG CACGCGTGGGTTCGAATCCCATCCTC GTCG

252	tRNA-Ser-GCT->tca--4-1	GACGAGGTGGCCGAGTGGTTAAGGCG ATGGACTtcaAATCCATTGTGCTCTG CACGCGTGGGTTCGAATCCCATCCTC GTCG
253	tRNA-Ser-GCT->tca--5-1	GATGAGGTGGCCGAGTGGTTAAGGCG ATGGACTtcaAATCCATTGTGCTCTG CACGCATGGGTTCGAATCCCATCCTC ATCG
254	tRNA-Ser-GCT->tta--1-1	GACGAGGTGGCCGAGTGGTTAAGGCG ATGGACTttaAATCCATTGTGCTCTG CACGCGTGGGTTCGAATCCCACCTTC GTCG
255	tRNA-Ser-GCT->tta--2-1	GACGAGGTGGCCGAGTGGTTAAGGCG ATGGACTttaAATCCATTGTGCTCTG CACGCATGGGTTCGAATCCCATCCTC GTCG
256	tRNA-Ser-GCT->tta--3-1	GACGAGGTGGCCGAGTGGTTAAGGCG ATGGACTttaAATCCATTGTGCTTTG CACGCGTGGGTTCGAATCCCATCCTC GTCG
257	tRNA-Ser-GCT->tta--4-1	GACGAGGTGGCCGAGTGGTTAAGGCG ATGGACTttaAATCCATTGTGCTCTG CACGCGTGGGTTCGAATCCCATCCTC GTCG
258	tRNA-Ser-GCT->tta--5-1	GATGAGGTGGCCGAGTGGTTAAGGCG ATGGACTttaAATCCATTGTGCTCTG CACGCATGGGTTCGAATCCCATCCTC ATCG
259	tRNA-Ser-GGA->cta--1-1	GCTGAAATAGCTCAGTTGGGAGAGCA TTAGACTctaGATCTAAAGGTCCCTG GTTTGATCCCGGGTTTCGGCA
260	tRNA-Ser-GGA->tca--1-1	GCTGAAATAGCTCAGTTGGGAGAGCA TTAGACTtcaGATCTAAAGGTCCCTG GTTTGATCCCGGGTTTCGGCA

261	tRNA-Ser-GGA->tta--1-1	GCTGAAATAGCTCAGTTGGGAGAGCA TTAGACTttaGATCTAAAGGTCCCTG GTTTGATCCCGGGTTTCGGCA
262	tRNA-Ser-TGA->cta--1-1	GCAGCGATGGCCGAGTGGTTAAGGCG TTGGACTctaAATCCAATGGGGTCTC CCCGCGCAGGTTCGAACCCTGCTCGC TGCG
263	tRNA-Ser-TGA->tca--1-1	GCAGCGATGGCCGAGTGGTTAAGGCG TTGGACTtcaAATCCAATGGGGTCTC CCCGCGCAGGTTCGAACCCTGCTCGC TGCG
264	tRNA-Ser-TGA->tta--1-1	GCAGCGATGGCCGAGTGGTTAAGGCG TTGGACTttaAATCCAATGGGGTCTC CCCGCGCAGGTTCGAACCCTGCTCGC TGCG
265	tRNA-Trp-CCA->cta--1-1	GACCTCGTGGCGCAATGGTAGCGCGT CTGACTctaGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
266	tRNA-Trp-CCA->cta--2-1	GACCTCGTGGCGCAACGGTAGCGCGT CTGACTctaGATCAGAAGGCTGCGTG TTCGAATCACGTCGGGGTCA
267	tRNA-Trp-CCA->cta--3-1	GGCCTCGTGGCGCAACGGTAGCGCGT CTGACTctaGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
268	tRNA-Trp-CCA->cta--4-1	GACCTCGTGGCGCAACGGTAGCGCGT CTGACTctaGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
269	tRNA-Trp-CCA->cta--5-1	GACCTCGTGGCGCAATGGTAGCGCGT CTGACTctaGATCAGAAGGTTGCGTG TTCAAGTCACGTCGGGGTCA
270	tRNA-Trp-CCA->cta--6-1	GACCTCGTGGCACAATGGTAGCACGT CTGACTctaGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA

271	tRNA-Trp-CCA->tca--1-1	GACCTCGTGGCGCAATGGTAGCGCGT CTGACTtcaGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
272	tRNA-Trp-CCA->tca--2-1	GACCTCGTGGCGCAACGGTAGCGCGT CTGACTtcaGATCAGAAGGCTGCGTG TTCGAATCACGTCGGGGTCA
273	tRNA-Trp-CCA->tca--3-1	GGCCTCGTGGCGCAACGGTAGCGCGT CTGACTtcaGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
274	tRNA-Trp-CCA->tca--4-1	GACCTCGTGGCGCAACGGTAGCGCGT CTGACTtcaGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
275	tRNA-Trp-CCA->tca--5-1	GACCTCGTGGCGCAATGGTAGCGCGT CTGACTtcaGATCAGAAGGTTGCGTG TTCAAGTCACGTCGGGGTCA
276	tRNA-Trp-CCA->tca--6-1	GACCTCGTGGCACAATGGTAGCACGT CTGACTtcaGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
277	tRNA-Tyr-GTA->cta--1-1	CCTTCGATAGCTCAGTTGGTAGAGCG GAGGACTctaGATCCTTAGGTCGCTG GTTCGAATCCGGCTCGAAGGA
278	tRNA-Tyr-GTA->cta--2-1	CCTTCGATAGCTCAGTTGGTAGAGCG GAGGACTctaGATCCTTAGGTCGCTG GTTCGATTCCGGCTCGAAGGA
279	tRNA-Tyr-GTA->cta--3-1	CCTTCGATAGCTCAGCTGGTAGAGCG GAGGACTctaGATCCTTAGGTCGCTG GTTCGATTCCGGCTCGAAGGA
280	tRNA-Tyr-GTA->cta--4-1	CCTTCGATAGCTCAGCTGGTAGAGCG GAGGACTctaGATCCTTAGGTCGCTG GTTCGACTCCGGCTCGAAGGA
281	tRNA-Tyr-GTA->cta--5-1	CTTTCGATAGTTCAGTTGGTAGAGCG GAGGACTctaGATCCTTAGGTCGCTG GTTCGAGTCCGGCTCGAAGGA

282	tRNA-Tyr-GTA->tta--1-1	CCTTCGATAGCTCAGTTGGTAGAGCG GAGGACTttaGATCCTTAGGTCGCTG GTTCGAATCCGGCTCGAAGGA
283	tRNA-Tyr-GTA->tta--2-1	CCTTCGATAGCTCAGTTGGTAGAGCG GAGGACTttaGATCCTTAGGTCGCTG GTTCGATTCCGGCTCGAAGGA
284	tRNA-Tyr-GTA->tta--3-1	CCTTCGATAGCTCAGCTGGTAGAGCG GAGGACTttaGATCCTTAGGTCGCTG GTTCGATTCCGGCTCGAAGGA
285	tRNA-Tyr-GTA->tta--4-1	CCTTCGATAGCTCAGCTGGTAGAGCG GAGGACTttaGATCCTTAGGTCGCTG GTTCGACTCCGGCTCGAAGGA
286	tRNA-Tyr-GTA->tta--5-1	CTTTCGATAGTTCAGTTGGTAGAGCG GAGGACTttaGATCCTTAGGTCGCTG GTTCGAGTCCGGCTCGAAGGA

[00216] The suppressor tRNAs are tested for PTC readthrough activity by flow cytometry in cell lines containing dual fluorescent readthrough reporters. These reporters include three copies of a red fluorescent protein (tdTomato), TEV protease, a linker region containing a PTC, and three copies of a green fluorescent protein (EGFP). A schematic of a reporter construct is shown in **FIGURE 3**. In the absence of any PTC readthrough as a result of a suppressor tRNA, translation will be terminated by the PTC within the linker region, and only tdTomato will be expressed (and therefore only red fluorescence detected). PTC readthrough activity as a result of a suppressor tRNA will allow translation to proceed through the PTC in the linker region, and for both tdTomato and EGFP to be expressed (and therefore both red and green fluorescence detected). Accordingly, readthrough can be assessed by quantifying the percentage of viable cells expressing both the red and green fluorescent reporters above background (double positive %).

Example 7

[00217] This Example describes glutamine aminoacylated suppressor tRNAs that facilitate read-through of a premature termination codon (PTC).

[00218] In this Example, all mature tRNA sequences were expressed in the context of upstream and downstream genomic flanking sequences (±200 bps) from tRNA-Gln-TTG-1-1 — a highly expressed glutamine-tRNA, i.e., the tRNA sequences were expressed with a 5' flanking

sequence of SEQ ID NO: 173 and a 3' flanking sequence of SEQ ID NO: 174. All mature tRNA sequences including upstream and downstream genomic flanking sequences were generated in a pGL4 vector backbone.

[00219] The Gln^{CTA} suppressor tRNAs were tested for PTC readthrough activity by flow
 5 cytometry in Flp-In-293 cells that either contain an integrated dual fluorescent readthrough reporter or were transiently co-transfected with an expression construct containing a dual fluorescent readthrough reporter. The reporters included three copies of a red fluorescent protein (tdTomato), TEV protease, a linker region containing a PTC, and three copies of a green fluorescent protein (EGFP). A schematic of the reporter construct is shown in **FIGURE 3**. In
 10 the absence of any PTC readthrough as a result of a suppressor tRNA, translation will be terminated by the PTC within the linker region, and only tdTomato will be expressed (and therefore only red fluorescence detected). PTC readthrough activity as a result of a suppressor tRNA allows translation to proceed through the PTC in the linker region, and for both tdTomato and EGFP to be expressed (and therefore both red and green fluorescence to be detected).
 15 Accordingly, readthrough was assessed with flow cytometry by quantifying the percentage of viable cells expressing both the red and green fluorescent reporters above background (double positive %). To screen for suppressor tRNAs with readthrough activity at PTCs relevant to Dravet syndrome, linker regions were generated containing the PTC and eight flanking codons on either side of the PTC from the SCN1A transcript of three patients with Gln(Q)-to-TAG
 20 nonsense mutations in SCN1A: patient 3 (W1397X), patient 4 (S1505X), and patient 5 (Q1810X).

[00220] The linker region derived from the patient 3 SCN1A transcript is as follows, and the reporter including this linker region is referred to as the patient3-Gln-TAG (W1397X) reporter:
 25 ATAGAAAGAAATGAGACTGCTCGATagAAAAATGTGAAAGTAACTTTGAT (SEQ ID NO: 889).

A corresponding linker with a wild-type Trp(W) codon in place of the PTC was used as a control, and had the following sequence:

ATAGAAAGAAATGAGACTGCTCGATGGAAAAATGTGAAAGTAACTTTGAT (SEQ ID NO: 890).

30 [00221] The linker region derived from the patient 4 SCN1A transcript is as follows, and the reporter including this linker region is referred to as the patient4-Gln-TAG (S1505X) reporter:

TATAATGCAATGAAAAAATTAGGAtagAAAAAACCGCAAAAGCCTATACCT (SEQ ID NO: 891).

A corresponding linker with a wild-type Ser(S) codon in place of the PTC was used as a control, and had the following sequence:

5 TATAATGCAATGAAAAAATTAGGATCGAAAAAACCGCAAAAGCCTATACCT (SEQ ID NO: 892).

[00222] The linker region derived from the patient 5 SCN1A transcript is as follows, and the reporter including this linker region is referred to as the patient5-Gln-TAG (Q1810X) reporter:

10 GAGAAGTTTGATCCCGATGCAACTtagTTCATGGAATTTGAAAAATTATCT (SEQ ID NO: 893).

A corresponding linker with a wild-type Gln codon in place of the PTC was used as a control, and had the following sequence:

15 GAGAAGTTTGATCCCGATGCAACTCAGTTCATGGAATTTGAAAAATTATCT (SEQ ID NO: 894).

[00223] The Gln^{CTA} suppressor tRNAs (SEQ ID NOs: 178-190) were tested for PTC readthrough activity by flow cytometry in multiple assay contexts, including (i) human Flp-In-293 cells transiently co-transfected with the patient3-Gln-TAG (W1397X) reporter, the patient4-Gln-TAG (S1505X) reporter, or the patient5-Gln-TAG (Q1810X) reporter (results are shown in 20 **FIGURE 24** and (ii) a human Flp-In-293 cell line cell line stably expressing the patient3-Gln-TAG (W1397X) reporter and transiently transfected with a plasmid encoding a Gln^{CTA} suppressor tRNA (results are shown in **FIGURE 25**). Transfections were done using the Lipofectamine 3000 Transfection Reagent according to the manufacturer's protocol.

[00224] Together, these results demonstrate that the described suppressor tRNAs can 25 facilitate expression of transcripts, *e.g.*, SCN1A transcripts, containing premature termination codons associated with disorders, *e.g.*, Dravet syndrome.

Example 8

[00225] This Example describes the impact of the nucleotide sequence flanking a 30 suppressor tRNA on the read-through of a premature termination codon (PTC) by the suppressor tRNA.

[00226] Expression vectors were generated that encoded an EGFP-R96X-TGA reporter (SEQ ID NO: 31, as described in Example 1) and a single copy of the Arg_{TCA} suppressor tRNA #115 (tRNA-Arg-TCT-2-1-TCA-SUP_no intron, SEQ ID NO: 18, as described in Example 1). The expression vectors included sequences immediately 5' and 3' to the tRNA coding sequence that were in each instance derived from the genomic DNA that is 5' and 3' to the mouse tRNA-Arg-TCG-1-1 gene but were of varying length. Details of the expression vectors are shown in **TABLE 11**.

TABLE 11

Name	5' to tRNA coding sequence	tRNA coding sequence	3' to tRNA coding sequence
Flank300	200 nt 5' to the mouse tRNA-Arg-TCG-1-1 gene (SEQ ID NO: 26)	TCA-115 (SEQ ID NO: 18)	104 nt 3' to the mouse tRNA-Arg-TCG-1-1 gene (SEQ ID NO: 32)
Flank20	20 nt 5' to the mouse tRNA-Arg-TCG-1-1 gene (SEQ ID NO: 895)	TCA-115 (SEQ ID NO: 18)	17 nt 3' to the mouse tRNA-Arg-TCG-1-1 gene (SEQ ID NO: 896)
Flank10	10 nt 5' to the mouse tRNA-Arg-TCG-1-1 gene (SEQ ID NO: 897)	TCA-115 (SEQ ID NO: 18)	17 nt 3' to the mouse tRNA-Arg-TCG-1-1 gene (SEQ ID NO: 896)
Flank0	Random sequence	TCA-115 (SEQ ID NO: 18)	17 nt 3' to the mouse tRNA-Arg-TCG-1-1 gene (SEQ ID NO: 896)

10 [00227] The expression vectors in **TABLE 11** were tested for PTC readthrough activity by flow cytometry in Neuro-2a cells. Transfections were performed using the Lipofectamine 3000 Transfection Reagent according to the manufacturer's protocol. The results are shown in **FIGUREs 26A** and **26B** and demonstrate that, although the Arg_{TCA} suppressor tRNA #115, exhibits activity even with a random 5' leader sequence, using a 5' leader sequence from an
15 endogenous tRNA gene can increase suppressor tRNA activity.

Example 9

[00228] This Example describes the impact of the nucleotide sequence flanking a suppressor tRNA on the read-through of a premature termination codon (PTC) by the suppressor tRNA.

[00229] A library of expression vectors was generated that included (i) one of 20 unique 100 nt leader sequences (a sequence immediately 5' to the tRNA coding sequence) derived from the human genomic DNA that is immediately 5' to an endogenous tRNA gene in combination with (ii) a nucleotide sequence encoding TCA-115 (tRNA-Arg-TCT-2-1-TCA-SUP_no intron, SEQ ID NO: 18, as described in Example 1) or TTA-163 (tRNA-Gln-TTG-3-1-TTA-SUP, SEQ ID NO: 45, as described in Example 4). A schematic illustrating the design of the expression vector constructs in the library is depicted in **FIGURE 27**. The 20 unique 100 nt leader sequences included sequences derived from the highest abundance tRNAs in human HEK293 cells and include leader sequences derived from Arg-TCT-1-1 (SEQ ID NO: 875), Tyr-GTA-5-1 (SEQ ID NO: 883), Ser-GCT-3-1 (SEQ ID NO: 878), Arg-TCG-1-1 (SEQ ID NO: 886), Arg-TCG-3-1 (SEQ ID NO: 888), Ser-TGA-1-1 (SEQ ID NO: 879), Arg-TCG-5-1 (SEQ ID NO: 871), Lys-TTT-6-1 (SEQ ID NO: 887), Asn-GTT-1-1 (SEQ ID NO: 880), Arg-CCG-2-1 (SEQ ID NO: 877), Ala-AGC-4-1 (SEQ ID NO: 874), Leu-TAA-1-1 (SEQ ID NO: 876), Ser-CGA-4-1 (SEQ ID NO: 870), Ser-TGA-4-1 (SEQ ID NO: 869), Ser-GCT-2-1 (SEQ ID NO: 872), Arg-TCT-2-1 (SEQ ID NO: 881), Thr-TGT-1-1 (SEQ ID NO: 885), Ile-AAT-4-1 (SEQ ID NO: 873), Val-CAC-2-1 (SEQ ID NO: 884), or Asn-GTT-3-1 (SEQ ID NO: 882) genes.

[00230] The leader sequences in combination with the Arg_{TCA} suppressor tRNA #115 (SEQ ID NO: 18) or the Gln_{TTA} suppressor tRNA #163 (SEQ ID NO: 45) were tested for PTC readthrough activity by flow cytometry in cell lines co-transfected with an EGFP-R96X-TGA reporter (SEQ ID NO: 31) for Arg_{TCA} constructs or an EGFP-Q69X-TAA reporter (SEQ ID NO: 175) for Gln_{TTA} constructs. The results are shown in **FIGURES 28-32** and indicate that (i) the activity of suppressor tRNAs is influenced by the leader sequence, and (ii) suppressor tRNAs (including suppressor tRNAs of different classes) when combined with the identified leader sequences showed high readthrough activity.

25 **Example 10**

[00231] This Example describes readthrough activity of certain suppressor tRNAs disclosed herein and small molecule nonsense suppression therapies.

[00232] The suppressor tRNAs were tested alongside nonsense suppression drugs translarna (ataluren), gentamicin, and G418 (geneticin). PTC readthrough activity was measured in Neuro-2a cells ~48 hours after transfection with an expression construct containing a CAG:NLS-EGFP (Q69X-TAA) reporter (as described in Example 4, SEQ ID NO: 175) and either (i) including a Gln suppressor tRNA (either # 002, tRNA-Gln-TTG-1-1-TTA-SUP, SEQ ID NO: 36, or #196, tRNA-Gln-TTG-1-1-CTA-SUP, SEQ ID NO: 178, both as described in Example 4) at the

indicated copy number on the same construct, or (ii) treated with ataluren, (iii) treated with gentamicin, or (iv) treated with G418. Transfections were performed using the Lipofectamine 3000 Transfection Reagent according to the manufacturer's protocol. For all experimental conditions, cell culture medium was replaced with fresh medium ~6 hours after transfection and the indicated drugs at the indicated concentrations were added at this point. PTC readthrough activity was measured as the percentage of EGFP positive cells as measured by flow cytometry. A reporter containing wildtype EGFP without a PTC was used as a control. Cell viability in cells receiving the same set of treatments was assessed by flow cytometry following staining with 7-Amino Actinomycin D (7-AAD; Thermo Fisher Scientific #006993-50), a membrane impermeant dye that is generally excluded from viable cells, which was used according to the manufacturer's protocol. Results are shown in **FIGURES 33-34** for the Gln^{TTA} suppressor tRNA #002 (SEQ ID NO: 36) and **FIGURES 35-36** for the Gln^{CTA} suppressor tRNA #196 (SEQ ID NO: 178). Together, the results demonstrate that the suppressor tRNAs produce greater readthrough than any of the nonsense suppression drugs. Additionally, the results show that, unlike for any of the nonsense suppression drugs, treatment with the suppressor tRNAs is not accompanied by a decrease in cell viability.

Example 11

[00233] This Example describes rescue of full-length SCN1a protein expression by certain suppressor tRNAs disclosed herein.

[00234] Flp-In-293 cells were (i) transfected with an expression construct containing mouse SCN1A with an Arg(R)-to-TGA PTC (R1407X) and a 3xFLAG tag peptide (DYKDHD-G-DYKDHD-I-DYKDDDDK) at the C-terminus (SEQ ID NO: 899) and (ii) either co-transfected with an expression construct containing the Arg^{TCA} suppressor tRNA #115 (tRNA-Arg-TCT-2-1-TCA-SUP_no intron, SEQ ID NO: 18), or treated with G418, gentamicin, or ataluren. An expression construct containing wild-type mouse SCN1A and a 3xFLAG tag peptide at the C-terminus (SEQ ID NO: 898) was used as a control. SEQ ID NOs: 898 and 899 are as follows:

ATGGAGCAAACAGTGCTTGTACCACCAGGACCTGACAGCTTCAACTTCTTCACCAGAGAATCCC
TTGCAGCTATTGAAAGGCGCATTCGAGAAGAGAAGGCTAAGAATCCCAAGCCAGACAAAAAGA
TGATGATGAAAATGGCCCAAAGCCAAACAGTGACTTGGAAGCTGGGAAGAACCTTCCATTTATC
TATGGAGACATTCCTCCAGAGATGGTGTCTGGAGCCTCTGGAGGACCTGGACCCCTACTATATCA
ATAAGAAGACTTTTATAGTATTGAATAAAGGGAAGGCCATCTTCCGGTTCAGTGCCACCTCCGC
CCTGTACATTTTAAACACCCTTCAATCCTCTTAGGAAAATAGCTATTAAGATTTTGGTACACTCA
TTATTCAGCATGTTAATCATGTGCACTATTTTGACAAACTGTGTATTTATGACAATGAGTAACC

CTCCCGACTGGACAAAGAATGTGGAGTACACCTTCACAGGAATATATACTTTTGAATCACTAAT
AAAAATTATTGCAAGGGGCTTCTGTTTAGAAGATTTTACTTTCCTTCGCGACCCATGGAAGTGG
CTGGACTTCACTGTCATTACATTTCGCATATGTGACGGAGTTTGTGGACCTGGGCAATGTCTCAG
CATTGAGAACATTCAGAGTTCTTCGAGCATTGAAAAC TATTTTCAGTCATTCCAGGCCTGAAGAC
5 CATCGTGGGGGCCCTGATCCAGTCGGTGAAGAAGCTGTCTGACGTCATGATACTCACTGTGTTC
TGTCTCAGTGTGTTTCGCACTCATCGGGTTGCAGCTCTTCATGGGCAACCTGAGGAATAAATGTG
TACAGTGGCCTCCCACCAACGCTTCCCTTGAGGAACATAGCATAGAGAAGAATATAACTATGGA
TTACAATGGCACACTTGTAATGAAACCGTGTTTCGAGTTTGACTGGAAATCATAATTCAAGAC
TCAAGATATCATTATTTCTGGAGGGTGTTTTAGATGCACTGCTGTGTGGAAATAGCTCTGATG
10 CAGGCCAATGTCCAGAAGGATATATGTGTGTAAAAGCTGGTAGAAACCCTAATTATGGTTACAC
AAGCTTTGATACCTTCAGTTGGGCATTTTTGTCCCTGTTTCGACTGATGACTCAGGACTTCTGG
GAAAATCTATACCAACTGACATTGCGTGCTGCTGGCAAAACCTACATGATATTTTTTTGTGCTGG
TCATTTTCTTGGGCTCATTCTACCTGATAAACTTGATCCTGGCTGTGGTGGCCATGGCCTATGA
GGAGCAGAATCAGGCCACACTGGAGGAGGCTGAACAGAAAGAGGCAGAATTTTCAGCAGATGTTG
15 GAGCAACTTAAGAAGCAGCAAGAGGCTGCACAGCAGGCAGCGGCTACAACAGCCTCAGAACATT
CCAGGGAGCCCAGTGCAGCAGGCAGGCTCTCAGATAGCTCTTCAGAAGCCTCTAAGTTGAGTTC
GAAGAGTGCTAAAGAAAGACGAAATCGGAGGAAAAAAGGAAACAGAAAGAGCAGTCTGGAGGA
GAAGAGAAAGATGATGATGAATTCCACAAGTCTGAGTCTGAAGACAGCATCAGGAGGAAGGGGT
TTCGCTTCTCCATAGAAGGGAATAGACTGACATATGAAAAGAGGTACTCTTCCCCGCATCAGTC
20 TCTGTTAAGCATTCGTGGTTCCTGTTCTCCCCAAGACGCAATAGCAGAACAAAGTCTTTTCAGC
TTTAGAGGGCGAGCCAAGGATGTGGGGTCTGAGAATGACTTTGCTGATGATGAACACAGCACCT
TTGAGGATAATGAGAGCCGTAGAGACTCACTGTTTCGTTCCCCGAAGACACGGAGAGCGACGCAA
CAGTAACCTGAGCCAGACCAGCAGGTCCTCCCGAATGCTGGCGGTGTTTCCAGCCAATGGGAAG
ATGCACAGCACGGTGGATTGCAATGGTGTGGTTTTCTTGGTTGGTGGACCCTCAGTTCCCACAT
25 CGCCAGTTGGACAGCTTCTGCCAGAGGGAACAACCACTGAAACTGAGATGAGAAAGAGGAGGTC
GAGCTCTTTCCATGTTTCCATGGACTTTCTAGAAGATCCTTCCCAGAGGCAAAGGGCAATGAGC
ATAGCCAGCATCTTAACAAATACAGTAGAAGAACTAGAAGAATCCAGGCAGAAATGTCCACCCT
GTTGGTATAAATTTTCCAACATATTCTTAATTTGGGACTGTTCTCCATATTGGCTGAAAGTTAA
ACATATTGTCAACCTGGTGGTGATGGACCCATTTGTTGATCTGGCCATTACCATCTGCATTGTG
30 TTAAATACGCTCTTCATGGCTATGGAGCACTACCCCATGACTGAACATTTCAACCATGTTCTTA
CAGTGGGAACTTGGTCTTCACTGGGATTTTCACAGCAGAAATGTTCTGAAAATCATCGCAAT
GGATCCTTACTATTACTTCCAAGAAGGCTGGAATATCTTTGATGGTTTCATTGTGACACTCAGC
CTGGTAGAACTTGGCCTTGCCAATGTGGAAGGATTGTCAGTTCTCCGTTTCAATTCGACTGCTCC
GAGTGTTCAAGTTGGCAAAGTCTTGGCCACACTGAATATGCTCATTAAGATCATTGGTAACTC
35 GGTGGGAGCACTGGGCAACCTGACTCTGGTGTGGCCATCATTGTCTTTATTTTTTGCCGTGGTT

GGCATGCAGCTGTTTGGAAAAAGTTACAAAGATTGTGTCTGCAAAATTGCCACTGACTGCAAAC
 TCCCACGTTGGCACATGAACGACTTCTTCCACTCGTTCCTGATCGTGTTCCGCGTGCTGTGTGG
 GGAGTGGATAGAGACCATGTGGGACTGCATGGAGGTGGCAGGACAAGCTATGTGCCTTACTGTC
 TTCATGATGGTCATGGTGATTGGGAACCTTGTGGTCTTGAACCTCTTTCTGGCCTTGCTTCTGA
 5 GCTCATTTAGTGCAGACAACCTTGCAGCCACTGATGATGACAATGAGATGAACAACCTGCAGAT
 TGCTGTGGACAGGATGCACAAAGGAATAGCTTATGTAAAAAGAAAAATATATGAATTCATTCAA
 CAATCCTTTGTAAAGAAACAGAAGATTCTAGATGAAATTAAGCCACTTGATGATCTAAACAACA
 GAAAAGACAATTGTATCTCTAACCACACAACAGAAATTGGGAAAGATCTGGACTGTCTGAAAGA
 TGTGAATGGAACCACAAGTGGCATAGGGACGGGCAGCAGTGTGGAGAAGTACATCATTGATGAG
 10 AGTGATTATATGTCATTCATAAACAACCCAGCCTCACTGTGACTGTGCCCATTGCTGTGGGAG
 AGTCTGACTTTGAGAACTTAAACACAGAAGACTTTAGCAGTGAATCAGATCTAGAAGAAAGCAA
 AGAGAAACTCAACGAAAGCAGTAGCTCCTCAGAGGGAAGCACAGTAGACATTGGGGCGCCTGCA
 GAGGAACAGCCTGTCATTGAACCAGAAGAAACCCTTGAGCCCGAAGCTTGCTTCACTGAAGGCT
 GTGTCCAGAGATTCAAGTGCTGTCAAATCAGCGTGGAAGAAGGAAGAGGGAAACAGTGGTGGAA
 15 CCTACGGAGGACGTGCTTCCGAATAGTTGAACACAACCTGGTTTGAGACCTTCATTGTGTTTCATG
 ATTCTCCTGAGTAGTGGTGCCCTGGCCTTTGAGGATATATATATATTGATCAGCGAAAGACGATCA
 AAACCATGCTGGAGTATGCTGACAAAGTCTTCACTTACATTTTCATCCTGGAGATGCTCCTCAA
 ATGGGTGGCCTATGGCTATCAAACATACTTCACCAATGCCTGGTGTGGCTAGACTTCTTAATT
 GTTGATGTTTCATTGGTCAGTTTAAACAGCAAATGCCTTGGGTACTCTGAACTCGGGGCCATCA
 20 AATCCCTAAGGACACTAAGAGCTCTGAGACCCCTAAGAGCCTTATCACGATTTGAAGGGATGAG
 GGTGGTTGTGAATGCCCTGTTAGGAGCAATTCCATCCATCATGAATGTGCTTCTGGTTTGCCTT
 ATATTCTGGCTAATTTTTCAGCATCATGGGCGTAAATTTGTTTGCTGGCAAATTCTACCACTGTG
 TTAACACCACAACCTGGTGACATATTTGAGATCAGCGAAGTCAATAATCATTCTGATTGCCTAAA
 ACTAATAGAAAGAAATGAGACCGCCCGGTGGAAAAATGTGAAAGTAAACTTTGATAATGTAGGA
 25 TTTGGGTATCTTTCTTTGCTTCAAGTTGCCACATTTAAGGGCTGGATGGATATCATGTATGCTG
 CAGTTGATTCCAGAAATGTTGAACTACAGCCTAAGTATGAGGAAAGCCTGTACATGTATTTGTA
 CTTCGTCATCTTCATCATCTTCGGGTCCTTCTTTACCCTGAACCTGTTTATTGGTGTTCATTATC
 GACAATTTCAACCAGCAAAAGAAGAAGTTTGGAGGTCAAGACATCTTTATGACAGAAGAACAGA
 AGAAATACTATAATGCAATGAAGAAATTAGGATCAAAAAAGCCACAAAAGCCTATCCCTCGACC
 30 TGGAACAAATTTCAAGGAATGGTTTTTGGACTTTGTAACCAGACAAGTGTTTGATATCAGCATC
 ATGATCCTCATCTGTCTGAACATGGTGACCATGATGGTGGAAACGGATGACCAGAGCGATTATG
 TGACAAGCATTTTGTACGCATCAACCTGGTGTTCATCGTCCTGTTACCGGCGAGTGTGTGCT
 CAAGCTCATCTCGCTCCGCCATTATTATTTACCATTGGATGGAACATTTTCGATTTTGTGGTG
 GTCATCCTCTCCATTGTAGGGATGTTTCTTGCGGAGCTAATAGAAAAGTATTTTGTGTCTCCTA
 35 CCCTGTTCCGAGTCATCCGCCTGGCCAGGATTGGACGAATCCTACGCCTGATCAAAGGTGCCAA

GGGGATCCGCACGCTGCTCTTTGCTCTGATGATGTCCCTTCCTGCGCTGTTTAACATCGGCCTC
CTGCTTTTTTCTCGTCATGTTTCTACGCCATCTTTGGGATGTCCAACCTTGCCTATGTTAAGA
GGGAAGTTGGGATTGATGACATGTTCAACTTTGAGACCTTCGGCAACAGCATGATCTGCCTGTT
CCAAATCACCACCTCTGCGGGCTGGGATGGACTGCTGGCCCCCATCCTCAACAGCAAACCCCT
5 GACTGTGACCCTAATAAAGTTAACCCTGGAAGCTCGGTGAAGGGAGACTGTGGGAACCCATCTG
TGGGGATTTTCTTTTTTGTCTAGCTACATCATATCCTTCCTGGTTGTGGTGAACATGTACAT
TGCTGTCATCCTGGAGAACTTCAGCGTTGCCACAGAAGAAAGTGCAGAGCCTCTGAGTGAGGAC
GACTTTGAGATGTTCTACGAGGTCTGGGAGAAGTTCGACCCTGACGCCACCCAGTTCATGGAAT
TTGAAAAATTATCTCAGTTTGCAGCTGCTCTAGAACCCCTCTCAATTTGCCACAACCAAACAA
10 ACTTCAGCTCATTGCCATGGACCTGCCCATGGTGAGTGGAGACCGCATCCACTGCCTGGACATC
TTATTTGCTTTTACAAAGCGGGTGTGGGTGAGAGTGGAGAGATGGATGCTCTTCGAATCCAGA
TGGAAGAGCGGTTCATGGCTTCCAACCCCTCCAAGGTCTCTTATCAGCCCATCACTACTACATT
AAAACGCAAACAAGAGGAGGTGTCAGCTGTTATCATTGAGCGAGCTTATAGGCGCCACCTTTTG
AAGCGAACAGTAAAACAAGCTTCATTCACATACAATAAGAACAACTCAAAGGTGGGGCTAATC
15 TTCTTGTAAGAAGACATGCTCATTGACAGAATAAACGAAAACCTCTATTACGGAGAAAACCTGA
CCTGACAATGTCCACAGCAGCTTGTCCGCCCTCCTACGATCGGGTGACAAAGCCAATCGTGGAG
AAACACGAGCAGGAAGGGAAGGATGAAAAAGCCAAAGGGAAAGACTACAAAGACCATGACGGTG
ATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGTAA (SEQ ID NO: 898); and
20 ATGGAGCAAACAGTGCTTGTACCACCAGGACCTGACAGCTTCAACTTCTTCACCAGAGAATCCC
TTGCAGCTATTGAAAGGCGCATTGCAGAAGAGAAGGCTAAGAATCCCAAGCCAGACAAAAAAGA
TGATGATGAAAATGGCCCAAAGCCAAACAGTGACTTGGAAGCTGGGAAGAACCTTCCATTTATC
TATGGAGACATTCCCTCCAGAGATGGTGTCTGGAGCCTCTGGAGGACCTGGACCCCTACTATATCA
ATAAGAAGACTTTTATAGTATTGAATAAAGGGAAGGCCATCTTCCGGTTCAGTGCCACCTCCGC
25 CCTGTACATTTTAAACACCCTTCAATCCTCTTAGGAAAATAGCTATTAAGATTTTGGTACACTCA
TTATTCAGCATGTTAATCATGTGCACTATTTTGACAACTGTGTATTTATGACAATGAGTAACC
CTCCCGACTGGACAAAGAATGTGGAGTACACCTTCACAGGAATATATACTTTTGAATCACTAAT
AAAAATTATTGCAAGGGGCTTCTGTTTAGAAGATTTTACTTTTCCTTCGCGACCCATGGAACCTGG
CTGGACTTCACTGTCATTACATTCGCATATGTGACGGAGTTTGTGGACCTGGGCAATGTCTCAG
30 CATTGAGAACATTCAGAGTTCTTCGAGCATTGAAAACCTATTTTCAGTCATTCCAGGCCTGAAGAC
CATCGTGGGGGCCCTGATCCAGTCGGTGAAGAAGCTGTCTGACGTCATGATACTCACTGTGTTC
TGTCTCAGTGTGTTTCGCACTCATCGGGTTGCAGCTCTTCATGGGCAACCTGAGGAATAAATGTG
TACAGTGGCCTCCCACCAACGCTTCCCTTGAGGAACATAGCATAGAGAAGAATATACTATGGA
TTACAATGGCACACTTGTAATGAAACCGTGTTTCGAGTTTGAAGTGGAAATCATACTTCAAGAC
35 TCAAGATATCATTATTTCTGGAGGGTGTTTTAGATGCACTGCTGTGTGGAAATAGCTCTGATG

CAGGCCAATGTCCAGAAGGATATATGTGTGTAAAAGCTGGTAGAAACCCTAATTATGGTTACAC
AAGCTTTGATACCTTCAGTTGGGCATTTTTGTCCCTGTTTCGACTGATGACTCAGGACTTCTGG
GAAAATCTATACCAACTGACATTGCGTGCTGCTGGCAAAACCTACATGATATTTTTTGTGCTGG
TCATTTTCTTGGGCTCATTCTACCTGATAAACTTGATCCTGGCTGTGGTGGCCATGGCCTATGA
5 GGAGCAGAATCAGGCCACACTGGAGGAGGCTGAACAGAAAGAGGCAGAATTCAGCAGATGTTG
GAGCAACTTAAGAAGCAGCAAGAGGCTGCACAGCAGGCAGCGGCTACAACAGCCTCAGAACATT
CCAGGGAGCCCAGTGCAGCAGGCAGGCTCTCAGATAGCTCTTCAGAAGCCTCTAAGTTGAGTTC
GAAGAGTGCTAAAGAAAGACGAAATCGGAGGAAAAAAGGAAACAGAAAGAGCAGTCTGGAGGA
GAAGAGAAAGATGATGATGAATTCCACAAGTCTGAGTCTGAAGACAGCATCAGGAGGAAGGGGT
10 TTCGCTTCTCCATAGAAGGGAATAGACTGACATATGAAAAGAGGTACTCTTCCCCGCATCAGTC
TCTGTTAAGCATTCGTGGTTCCTGTTCTCCCCAAGACGCAATAGCAGAACAAGTCTTTTCAGC
TTTAGAGGGCGAGCCAAGGATGTGGGGTCTGAGAATGACTTTGCTGATGATGAACACAGCACCT
TTGAGGATAATGAGAGCCGTAGAGACTCACTGTTTCGTTCCCCGAAGACACGGAGAGCGACGCAA
CAGTAACCTGAGCCAGACCAGCAGGTCCTCCCGAATGCTGGCGGTGTTTCCAGCCAATGGGAAG
15 ATGCACAGCACGGTGGATTGCAATGGTGTGGTTTCCTTGGTTGGTGGACCCTCAGTTCCCACAT
CGCCAGTTGGACAGCTTCTGCCAGAGGGAACAACCACTGAAACTGAGATGAGAAAGAGGAGGTC
GAGCTCTTTCCATGTTTCCATGGACTTTCTAGAAGATCCTTCCCAGAGGCAAAGGGCAATGAGC
ATAGCCAGCATCTTAACAAATACAGTAGAAGAACTAGAAGAATCCAGGCAGAAATGTCCACCCT
GTTGGTATAAATTTTCCAACATATTCTTAATTTGGGACTGTTCTCCATATTGGCTGAAAGTTAA
20 ACATATTGTCAACCTGGTGGTGATGGACCCATTTGTTGATCTGGCCATTACCATCTGCATTGTG
TTAAATACGCTCTTCATGGCTATGGAGCACTACCCCATGACTGAACATTTCAACCATGTTCTTA
CAGTGGGAAACTTGGTCTTCACTGGGATTTTTCACAGCAGAAATGTTCCCTGAAAATCATCGCAAT
GGATCCTTACTATTACTTCCAAGAAGGCTGGAATATCTTTGATGGTTTCATTGTGACACTCAGC
CTGGTAGAACTTGGCCTTGCCAATGTGGAAGGATTGTCAGTTCTCCGTTTCAATTCGACTGCTCC
25 GAGTGTTCAAGTTGGCAAAGTCTTGGCCCACACTGAATATGCTCATTAAGATCATTGGTAACTC
GGTGGGAGCACTGGGCAACCTGACTCTGGTGTGGCCATCATTGTCTTTATTTTTGCCGTGGTT
GGCATGCAGCTGTTTGGAAAAAGTTACAAAGATTGTGTCTGCAAAATTGCCACTGACTGCAAAC
TCCCACGTTGGCACATGAACGACTTCTTCCACTCGTTCCTGATCGTGTTCCGCGTGCTGTGTGG
GGAGTGGATAGAGACCATGTGGGACTGCATGGAGGTGGCAGGACAAGCTATGTGCCTTACTGTC
30 TTCATGATGGTCATGGTGATTGGGAACCTTGTGGTCTTGAACCTCTTTCTGGCCTTGCTTCTGA
GCTCATTTAGTGCAGACAACCTTGCAGCCACTGATGATGACAATGAGATGAACAACCTGCAGAT
TGCTGTGGACAGGATGCACAAAGGAATAGCTTATGTAAAAAGAAAAATATATGAATTCATTCAA
CAATCCTTTGTTAAGAAACAGAAGATTCTAGATGAAATTAAGCCACTTGATGATCTAAACAACA
GAAAAGACAATTGTATCTCTAACCACACAACAGAAATTGGGAAAGATCTGGACTGTCTGAAAGA
35 TGTGAATGGAACCACAAGTGGCATAGGGACGGGCAGCAGTGTGGAGAAGTACATCATTGATGAG

AGTGATTATATGTCATTCATAAACAACCCCAGCCTCACTGTGACTGTGCCCATTGCTGTGGGAG
AGTCTGACTTTGAGAACTTAAACACAGAAGACTTTAGCAGTGAATCAGATCTAGAAGAAAGCAA
AGAGAAACTCAACGAAAGCAGTAGCTCCTCAGAGGGAAGCACAGTAGACATTGGGGCGCCTGCA
GAGGAACAGCCTGTCATTGAACCAGAAGAAACCCTTGAGCCCGAAGCTTGCTTCACTGAAGGCT
5 GTGTCCAGAGATTCAAGTGCTGTCAAATCAGCGTGGAAGAAGGAAGAGGGAAACAGTGGTGGAA
CCTACGGAGGACGTGCTTCCGAATAGTTGAACACAACCTGGTTTGAGACCTTCATTGTGTTCATG
ATTCTCCTGAGTAGTGGTGCCCTGGCCTTTGAGGATATATATATTGATCAGCGAAAGACGATCA
AAACCATGCTGGAGTATGCTGACAAAGTCTTCACTTACATTTTCATCCTGGAGATGCTCCTCAA
ATGGGTGGCCTATGGCTATCAAACATACTTCACCAATGCCTGGTGTGGCTAGACTTCTTAATT
10 GTTGATGTTTCATTGGTCAGTTTAAACAGCAAATGCCTTGGGTACTCTGAACTCGGGGCCATCA
AATCCCTAAGGACACTAAGAGCTCTGAGACCCCTAAGAGCCTTATCACGATTTGAAGGGATGAG
GGTGGTTGTGAATGCCCTGTTAGGAGCAATTCCATCCATCATGAATGTGCTTCTGGTTTGCCTT
ATATTCTGGCTAATTTTCAGCATCATGGGCGTAAATTTGTTTGCTGGCAAATTCTACCACTGTG
TTAACACCACAACCTGGTGACATATTTGAGATCAGCGAAGTCAATAATCATTCTGATTGCCTAAA
15 ACTAATAGAAAGAAATGAGACCGCCTGATGGAAAAATGTGAAAGTAAACTTTGATAATGTAGGA
TTTGGGTATCTTTCTTTGCTTCAAGTTGCCACATTTAAGGGCTGGATGGATATCATGTATGCTG
CAGTTGATTCCAGAAATGTTGAACTACAGCCTAAGTATGAGGAAAGCCTGTACATGTATTTGTA
CTTCGTCATCTTCATCATCTTCGGGTCCTTCTTTACCCTGAACCTGTTTATTGGTGTCAATTATC
GACAATTTCAACCAGCAAAAGAAGAAGTTTGGAGGTCAAGACATCTTTATGACAGAAGAACAGA
20 AGAAATACTATAATGCAATGAAGAAATTAGGATCAAAAAAGCCACAAAAGCCTATCCCTCGACC
TGGAACAAATTTCAAGGAATGGTTTTTTGACTTTGTAACCAGACAAGTGTTTGATATCAGCATC
ATGATCCTCATCTGTCTGAACATGGTGACCATGATGGTGGAAACGGATGACCAGAGCGATTATG
TGACAAGCATTTTGTACGCATCAACCTGGTGTTTCATCGTCCTGTTACCCGGCGAGTGTGTGCT
CAAGCTCATCTCGCTCCGCCATTATTATTTACCATTGGATGGAACATTTTCGATTTTGTGGTG
25 GTCATCCTCTCCATTGTAGGGATGTTTCTTGCGGAGCTAATAGAAAAGTATTTTGTGTCTCCTA
CCCTGTTCCGAGTCATCCGCCTGGCCAGGATTGGACGAATCCTACGCCTGATCAAAGGTGCCAA
GGGGATCCGCACGCTGCTCTTTGCTCTGATGATGTCCCTTCCTGCGCTGTTTAACATCGGCCTC
CTGCTTTTTCTCGTCATGTTTCATCTACGCCATCTTTGGGATGTCCAACCTTGCCTATGTTAAGA
GGGAAGTTGGGATTGATGACATGTTCAACTTTGAGACCTTCGGCAACAGCATGATCTGCCTGTT
30 CCAAATCACCACTCTGCGGGCTGGGATGGACTGCTGGCCCCCATCCTCAACAGCAAACCCCT
GACTGTGACCCTAATAAAGTTAACCCTGGAAGCTCGGTGAAGGGAGACTGTGGGAACCCATCTG
TGGGGATTTTCTTTTTTGTGAGCTACATCATCATATCCTTCCTGGTTGTGGTGAACATGTACAT
TGCTGTCATCCTGGAGAACTTCAGCGTTGCCACAGAAGAAAGTGCAGAGCCTCTGAGTGAGGAC
GACTTTGAGATGTTCTACGAGGTCTGGGAGAAGTTTCGACCCTGACGCCACCCAGTTCATGGAAT
35 TTGAAAAATTATCTCAGTTTGCAGCTGCTCTAGAACCCCTCTCAATTTGCCACAACCAAACAA

ACTTCAGCTCATTGCCATGGACCTGCCCATGGTGAGTGGAGACCGCATCCACTGCCTGGACATC
 TTATTTGCTTTTACAAAGCGGGTGTGGGTGAGAGTGGAGAGATGGATGCTCTTCGAATCCAGA
 TGAAGAGCGGTTTCATGGCTTCCAACCCCTCCAAGGTCTCTTATCAGCCCATCACTACTACATT
 AAAACGCAAACAAGAGGAGGTGTCAGCTGTTATCATTAGCGAGCTTATAGGCGCCACCTTTTG
 5 AAGCGAACAGTAAAACAAGCTTCATTCACATACAATAAGAACAACAACTCAAAGGTGGGGCTAATC
 TTCTTGTAAGAAGACATGCTCATTTGACAGAATAAACGAAAACCTCTATTACGGAGAAAACCTGA
 CCTGACAATGTCCACAGCAGCTTGTCCGCCCTCCTACGATCGGGTGACAAAGCCAATCGTGGAG
 AAACACGAGCAGGAAGGGAAGGATGAAAAAGCCAAAGGGAAGACTACAAAGACCATGACGGTG
 ATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGTAA (SEQ ID NO: 899).

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[00235] SCN1A was detected by Western blot as follows. Protein was isolated at 24 hours post transfection in RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific #89900) containing Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific #87786) according to the manufacturer's protocol. Protein concentrations were determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific #23225). 30 µg of protein was separated on either NuPAGE 4-12% Bis-Tris (Thermo Fisher Scientific #NP0322BOX) or NuPAGE 3-8%, Tris-Acetate (Thermo Fisher Scientific #EA0375BOX) protein gels at 150V for 1.5 hours and transferred to PVDF membranes at 30V overnight followed by 250 mA for 30 min at 4°C. Blots were blocked in SuperBlock T20 Blocking Buffer (Thermo Fisher Scientific #37536) at room temperature for 1 hour, incubated with the primary anti-FLAG M2 antibody (Sigma, F1804-200UG, 1:1000) in TBST overnight at 4°C, washed 3 times with TBST, and incubated with the Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP secondary antibody (Thermo Fisher Scientific #31431, 1:30,000) at room temperature for 1 hour. The blots were developed by applying the SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific #34094) and signals were detected by the iBright Imaging system. The results are shown in **FIGURE 37** and demonstrate that the Arg^{TCA} suppressor tRNA #115, but not the small molecules drugs, was able to rescue full-length SCN1A protein expression.

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[00236] Flp-In-293 cells were also co-transfected with (i) an expression construct containing mouse SCN1A with an Arg(R)-to-TGA PTC (R1407X) and a 3xFLAG tag peptide at the C-terminus (SEQ ID NO: 899) and (ii) an expression construct containing the Arg^{TCA} suppressor tRNA #104 (tRNA-Arg-CCG-3-1-TCA-SUP, SEQ ID NO: 6), the Arg^{TCA} suppressor tRNA #106 (tRNA-Arg-CCT-2-1-TCA-SUP, SEQ ID NO: 8), or the Arg^{TCA} suppressor tRNA #115 (tRNA-Arg-TCT-2-1-TCA-SUP_no intron, SEQ ID NO: 18). SCN1A expression was measured by Western blot using an anti-FLAG antibody as described above in this Example.

30

The results are shown in **FIGURE 38** and demonstrate that each suppressor tRNA tested rescued full-length SCN1A protein expression.

[00237] Flp-In-293 cells were also co-transfected with (i) an expression construct containing mouse SCN1A with an Arg(R)-to-TGA PTC (R1407X) and a 3xFLAG tag peptide at the C-terminus (SEQ ID NO: 899) and (ii) an expression construct containing the Arg^{TCA} suppressor tRNA #115 (tRNA-Arg-TCT-2-1-TCA-SUP_no intron, SEQ ID NO: 18) at a range of doses (13 ng per well, 40 ng per well, 113 ng per well, or 400 ng per well; 6-well cell culture plate). SCN1A expression was measured by Western blot using an anti-FLAG antibody as described above in this Example. The results are shown in **FIGURE 39** and demonstrate that tRNA 115 was able to rescue full-length SCN1A protein expression over a broad dose range.

Example 12

[00238] This Example describes readthrough activity of disclosed suppressor tRNAs delivered by adeno-associated virus (AAV) vectors.

[00239] Constructs that were packaged into AAV-PHP.eB capsids are depicted in **FIGURE 40**. Construct 262 contains wild-type EGFP driven by an EF1a promoter. Construct 269 contains EGFP-R96X-TGA driven by an EF1a promoter (SEQ ID NO: 177) and two copies of the Arg^{TCA} suppressor tRNA #115 (tRNA-Arg-TCT-2-1-TCA-SUP_no intron, SEQ ID NO: 18, as described in Example 1) in the context of 55 bps upstream flanking genomic DNA from tRNA-Tyr-GTA-5-1 (SEQ ID NO: 900). Both constructs contain 5' and 3' ITR sequences from AAV2, which provide cis-acting elements for AAV replication and packaging. AAV-PHP.eB containing construct 262 and construct 269 was produced by Vigene Biosciences.

[00240] Prior to AAV transduction, 293 cells (Agilent #240073) were pre-transfected with an expression construct containing the LY6A gene (CCDS ID 27540.1) driven by the CMV early enhancer/chicken β actin (CAG) promoter, which is required for robust transduction by AAV-PHP.eB. For pre-transfection, cells were transiently transfected with the LY6A expression construct using lipofectamine 3000 Transfection Reagent according to the manufacturer's protocol. At ~24 hours post-transfection the media was exchanged for fresh media and the cells were allowed to recover for another 24 hours prior to viral transduction. The cells were then transduced at an MOI of 1E5 vg/cell. Results are depicted in **FIGURE 41**. AAV delivered Arg^{TCA} suppressor tRNA #115 resulted in ~13.2% PTC readthrough based on GFP intensity. The suppressor tRNA shows comparable readthrough activity when delivered by AAV or transient transfection.

Example 13

[00241] This Example describes ribosome profiling experiments that demonstrate that disclosed suppressor tRNAs do not cause a significant amount of off-target native stop codon read-through.

5 [00242] In order to determine whether suppressor tRNAs cause readthrough of native stop codons, ribosome profiling was used to quantify the number of ribosomes found in the 3' UTR of mRNAs from (i) cells transfected with an expression construct containing a suppressor tRNA relative to (ii) cells transfected with an expression construct that lacks a suppressor tRNA. Ribosomes typically terminate translation upon encountering a stop codon, so if suppressor
10 tRNAs cause increased readthrough of native stop codons, this will be indicated by an increase in the density of ribosomes found in the 3' UTR of mRNAs in cells expressing a suppressor tRNA, especially in the 3' UTR mRNAs containing a native stop codon that is recognized by the expressed suppressor tRNA.

[00243] Neuro-2a cells were transfected with either (i) an expression construct containing
15 an EGFP-R96X-TGA reporter (SEQ ID NO: 177) and the Arg_{TCA} suppressor tRNA #001 (SEQ ID NO: 11) on the same construct or (ii) an expression construct lacking a suppressor tRNA and containing a wild-type version of the EGFP reporter. Expression of EGFP is depicted in **FIGURE 42**. At ~48 hours post transfection, cells were subjected to ribosome footprint profiling as follows. Cells were lysed in the lysis buffer (10 mM Tris-HCl pH7.5, 5 mM MgCl₂,
20 100 mM KCl, 1% Triton X-100, 1 Mm DTT, 50 µg/mL Emetine (Sigma #324693), and 500 U/mL RNasin (Promega #N2615)) and cell lysates were sheared ten times with a 25-gauge needle followed by centrifugation at 20,000 g for 10 minutes at 4°C. The supernatants were digested with micrococcal nuclease (MNase; 120 units/OD A260 lysates; New England Biolabs #M0247S) at room temperature for 30 minutes prior to adding 5 uL SuperAse-IN (Thermo
25 Fisher Scientific #AM2694) to stop the reaction. MNase-treated extracts were loaded to a 15-45% sucrose gradient and separated by density in a SW 41Ti swinging-bucket rotor (Beckman Coulter #331362) at 41,000 rpm at 4°C for 2:26 hours. After fractionation and collection of fractions containing monosomes, ribosome protected mRNA fragments were precipitated from the sucrose overnight at -20°C with 1.25 mL of 95% ethanol. mRNA fragments were re-
30 suspended with 10 mM Tris-HCl, pH 8.0, and separated on a 15% denaturing polyacrylamide gel (TBE-urea gel; Thermo Fisher Scientific #EC68852BOX). RNA fragments with sizes ranging from 26 to 34 nt were excised from the gel and isolated to generate the ribosome-protected fragment library. After 3' linker ligation, rRNA depletion with the Ribo-Zero reagents in the

TruSeq Stranded Total RNA Library Prep Gold Kit (Illumina #20020598), reverse transcription, circularization, and PCR amplification with indexing primers, the PCR products were separated on an 8% nondenaturing polyacrylamide gel (Thermo Fisher Scientific #EC62152BOX). The barcoded cDNA libraries were extracted from the gel and sequenced by the NextSeq 550
5 Sequencing System with the single read run type. Following sequencing, raw reads were trimmed of adapters using Trimmomatic then depleted for non-coding RNA by aligning against Ensembl's mouse mm10 ncRNA reference using bowtie2. Remaining reads were aligned against UCSC's mm10 mouse reference assembly, again using bowtie2. Multi-mapping reads were discarded. The resulting final set of aligned reads was quantified using the RiboProfiling
10 package in R and custom Python scripting. Python was used to generate plots examining 3' UTR occupancy and fold change for each gene with 20 or more uniquely mapping reads, and the distributions for genes with each native stop codon were compared using the 2 sample Kolmogorov-Smirnov test. The results are depicted in **FIGURE 43**.

[00244] Together, these results demonstrate that suppressor tRNAs can facilitate expression
15 of transcripts, *e.g.*, EGFP-R96X-TGA, containing premature termination codons while not causing a significant amount of off-target native stop codon read-through in the expressing cells.

INCORPORATION BY REFERENCE

[00245] The entire disclosure of each of the patent and scientific documents referred to herein is incorporated by reference for all purposes.

20 EQUIVALENTS

[00246] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing
25 description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein

WHAT IS CLAIMED IS:

1. A tRNA comprising a nucleotide sequence set forth in Table 2.
2. The tRNA of claim 1, wherein the tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 19-21, 37, 39, 40, 44, 179, 181, 182, and 186.
3. The tRNA of claim 1 or 2, wherein the tRNA comprises a naturally occurring nucleotide modification.
4. The tRNA of any one of claims 1-3, wherein the 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.
5. An expression vector comprising a nucleotide sequence encoding the tRNA of any one of claims 1-4.
6. The expression vector of claim 5, wherein the expression vector comprises 1, 2, 3, 4, or more than 4 copy numbers of the nucleotide sequence encoding the tRNA.
7. The expression vector of claim 5 or 6, wherein the expression vector further comprises a nucleotide sequence set forth in Table 4.
8. The expression vector of claim 7, wherein the expression vector comprises a nucleotide sequence selected from SEQ ID NOs: 869-888.
9. An expression vector comprising a nucleotide sequence encoding a tRNA set forth in Table 3, wherein the expression vector comprises 1, 2, 3, 4, or more than 4 copy numbers of the nucleotide sequence encoding the tRNA.
10. An expression vector comprising a nucleotide sequence encoding a tRNA set forth in Table 3, wherein the expression vector further comprises a nucleotide sequence set forth in Table 4.
11. The expression vector of claim 9 or 10, wherein the tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 22, 35, 36, 38, 45, 178, 180, and 187.
12. The expression vector of claim 10 or 11, wherein the expression vector comprises a nucleotide sequence selected from SEQ ID NOs: 869-888.
13. The expression vector of any one of claims 5-12, wherein the expression vector is a viral vector.
14. The expression vector of claim 13, wherein the viral vector is a DNA virus vector.

15. The expression vector of claim 13 or 14, wherein the viral vector is an adeno-associated virus (AAV) vector.
16. A pharmaceutical composition comprising the tRNA of any one of claims 1-4 or the expression vector of any one of claims 5-15 and a pharmaceutically acceptable excipient.
17. The pharmaceutical composition of claim 16, wherein the tRNA or expression vector is not conjugated to or associated with another moiety.
18. The pharmaceutical composition of claim 17, wherein the tRNA or expression vector is not conjugated to or associated with a carrier particle.
19. The pharmaceutical composition of claim 8, wherein the carrier particle is an aminolipid particle.
20. The pharmaceutical composition of any one of claims 15-19, wherein the composition does not comprise a nanoparticle.
21. The pharmaceutical composition of any one of claims 15-20, wherein the composition does not comprise an aminolipid delivery compound.
22. A method of expressing in a mammalian cell a functional gene product encoded by a gene containing a premature termination codon, the method comprising introducing into the cell an effective amount of the tRNA of any one of claims 1-4 or the expression vector of any one of claims 5-15, 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.
23. The method of claim 22, wherein the cell contains less truncated gene product than a cell without the tRNA.
24. The method of claim 22 or 23, wherein the cell contains a greater amount of functional gene product than a cell without the tRNA.
25. The method of any one of claims 22-24, wherein the gene is a gene set forth in Table 5 or Table 6.
26. The method of claim 25, wherein the gene is a gene set forth in Table 5.
27. A method of expressing in a mammalian cell a functional gene product encoded by a gene containing a premature termination codon, the method comprising introducing into the cell an effective amount of a tRNA set forth in Table 3, or an expression vector comprising a nucleotide sequence encoding the tRNA, 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, wherein the gene is a gene set forth in Table 5.
28. The method of claim 27, wherein the tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 22, 35, 36, 38, 45, 178, 180, and 187.
 29. The method of any one of claims 22-28, wherein the gene is a SCN1A gene.
 30. A method of increasing in a cell voltage-gated sodium channel activity encoded by a SCN1A gene containing a premature termination codon, the method comprising introducing into the cell an effective amount of the tRNA of any one of claims 1-4 or the expression vector of any one of claims 5-15, thereby permitting an amino acid to be incorporated into the SCN1A gene product at a position that would otherwise result in a truncated SCN1A gene product caused by the premature termination codon.
 31. A method of increasing in a cell voltage-gated sodium channel activity encoded by a SCN1A gene containing a premature termination codon, the method comprising introducing into the cell an effective amount of a tRNA set forth in Table 3, or an expression vector comprising a nucleotide sequence encoding the tRNA, thereby permitting an amino acid to be incorporated into the SCN1A gene product at a position that would otherwise result in a truncated SCN1A gene product caused by the premature termination codon.
 32. The method of claim 31, wherein the tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 22, 35, 36, 38, 45, 178, 180, and 187.
 33. The method of any one of claims 29-32, wherein the SCN1A gene product produced with the tRNA is a functional SCN1A gene product.
 34. The method of claim 33, wherein the functional SCN1A gene product has greater activity than the truncated SCN1A gene product.
 35. The method of claim 33 or 34, wherein the functional SCN1A gene product is the Na_v1.1 protein.
 36. The method of any one of claims 33-35, wherein the functional SCN1A gene product comprises any one of SEQ ID NOs: 863-868.
 37. The method of claim 36, wherein the functional SCN1A gene product comprises SEQ ID NO: 863 or SEQ ID NO: 864.

38. The method of any one of claims 22-37, wherein the cell is a human cell.
39. The method of any one of claims 22-38, wherein the tRNA becomes aminoacylated in the cell.
40. A method of treating a premature termination codon-mediated disorder in a subject in need thereof wherein the subject has a gene with a premature termination codon, the method comprising administering to the subject an effective amount of the tRNA of any one of claims 1-4 or the expression vector of any one of claims 5-15, thereby to treat the disorder in the subject.
41. The method of claim 40, wherein the disorder is a disorder set forth in Table 5 or Table 6.
42. The method of claim 41, wherein the disorder is a disorder set forth in Table 5.
43. A method of treating a premature termination codon-mediated disorder in a subject in need thereof wherein the subject has a gene with a premature termination codon, the method comprising administering to the subject an effective amount of a tRNA set forth in Table 3, or an expression vector comprising a nucleotide sequence encoding the tRNA, thereby to treat the disorder in the subject, wherein the disorder is a disorder set forth in Table 5.
44. The method of claim 43, wherein the tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 22, 35, 36, 38, 45, 178, 180, and 187.
45. The method of any one of claims 40-44, wherein the disorder is Dravet syndrome.
46. The method of claim 45, wherein the method further comprises administering stiripentol, cannabidiol, a ketogenic diet, clobazam, topiramate, fenfluramine, or valproic acid to the subject.
47. The method of claim 45 or 46, wherein the gene is SCN1A.
48. The method of any one of claims 40-47, wherein the subject is a human.
49. The method of any one of claims 29-39 or 47, wherein the premature termination codon in the SCN1A gene is caused by a mutation selected from c.664C>T, c.1129C>T, c.1492A>T, c.1624C>T, c.1738C>T, c.1837C>T, c.2134C>T, c.2593C>T, c.3637C>T, c.3733C>T, c.3985C>T, c.4573C>T, c.5656C>T, and c.5734C>T.
50. The method of claim 49, wherein the premature termination codon in the SCN1A gene is caused by a mutation selected from c.1738C>T and c.3985C>T.

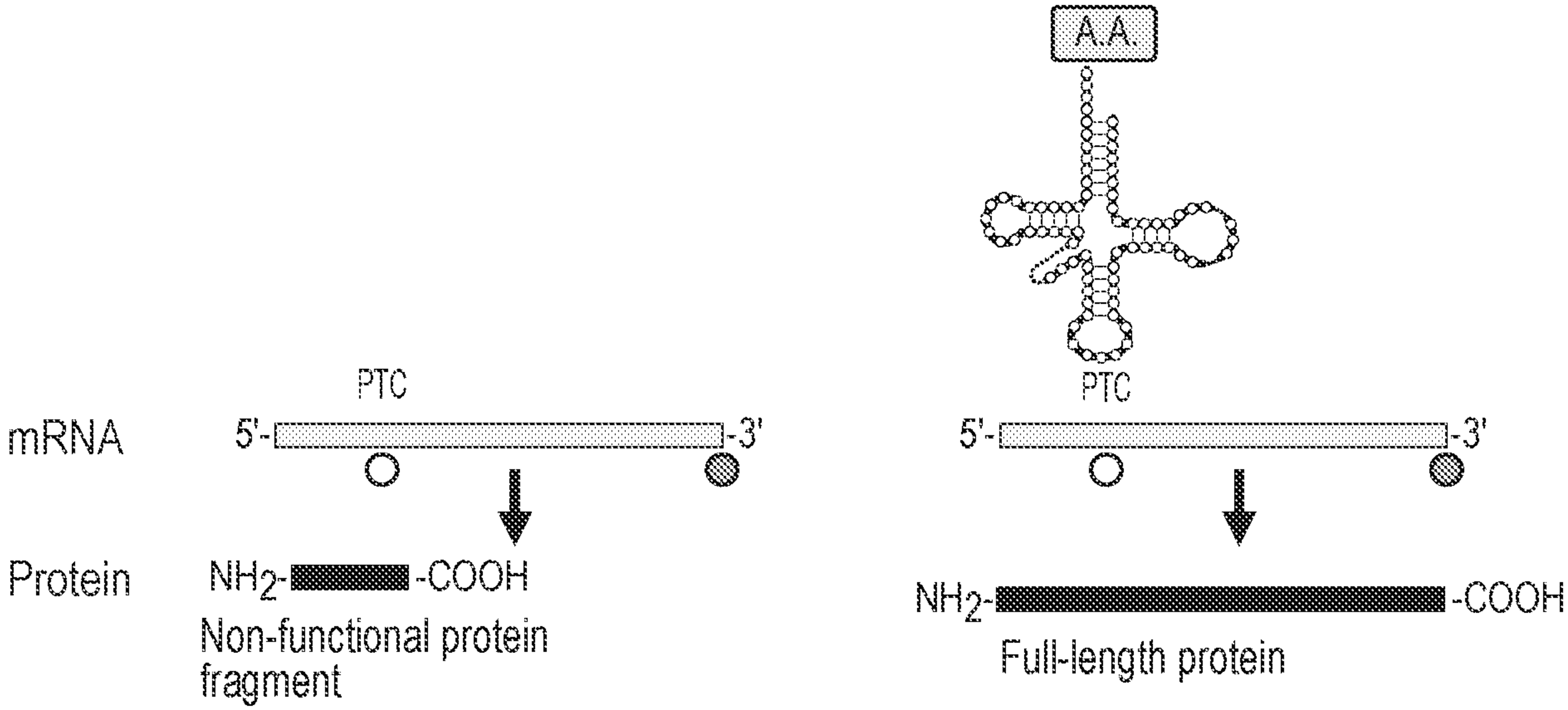


FIGURE 1

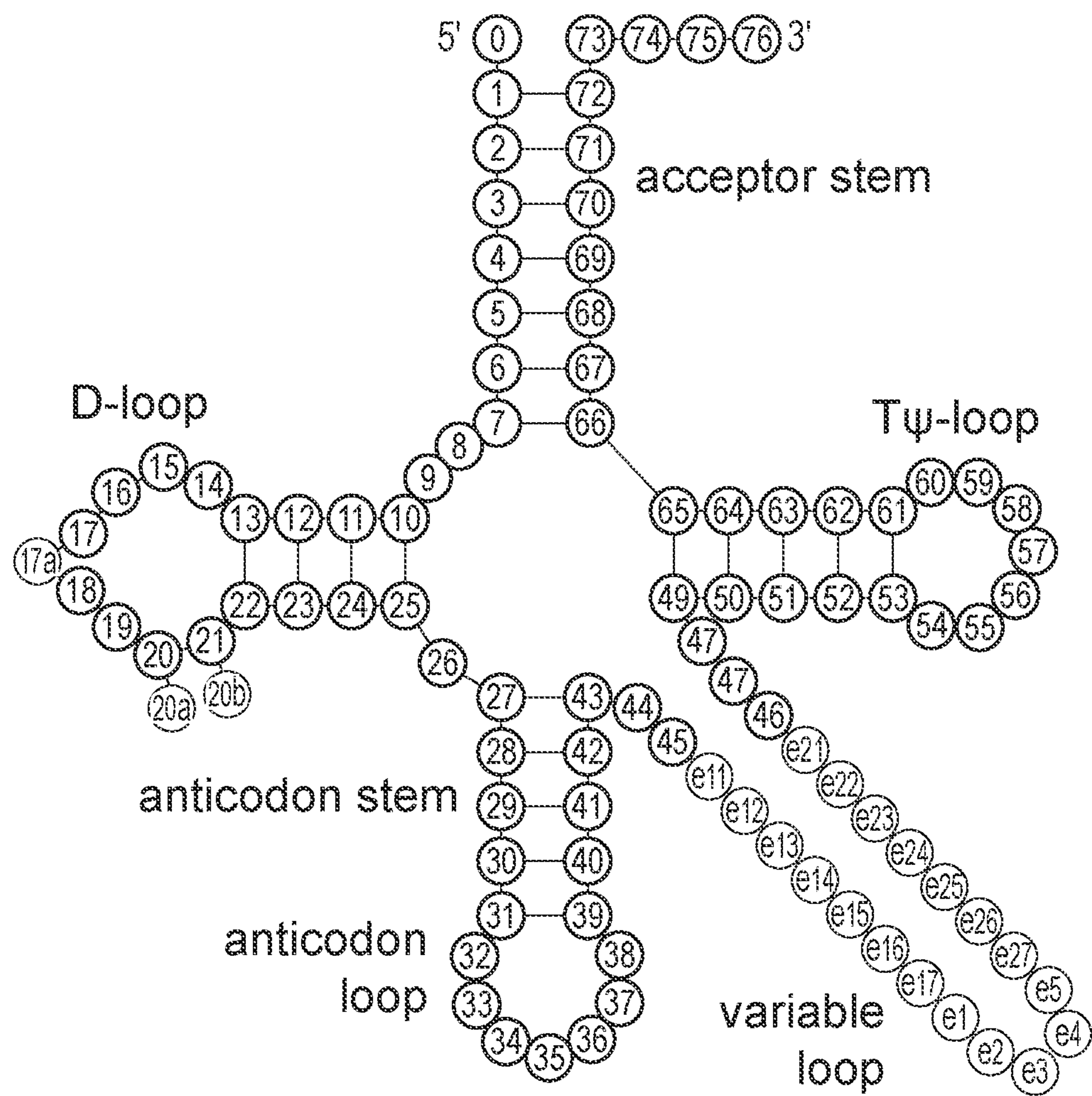
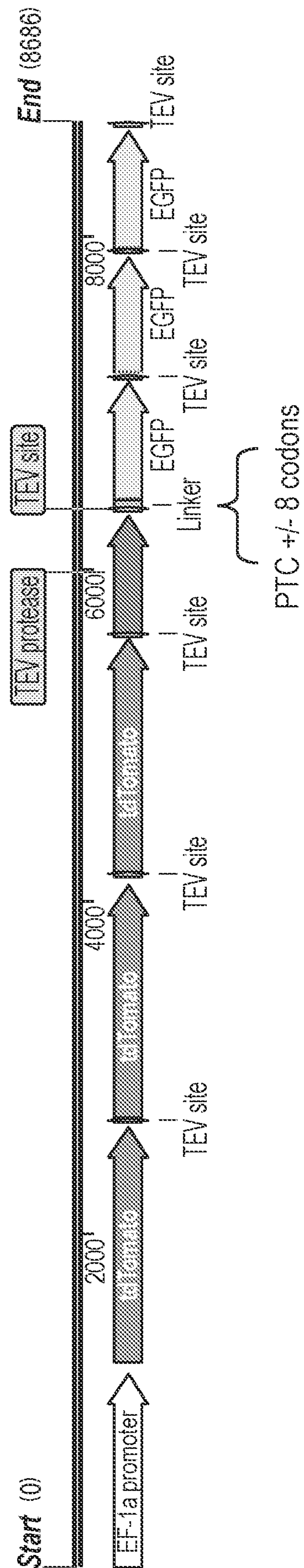


FIGURE 2A

3/49

position	residues	ratios	position	residues	ratios
0	Gm/G	1/4	35	ψ/U	7/50
1	ψ/U	3/14	36	ψ/xU/U	2/3/50
4	Am/A	2/28	37	m ¹ l/ms ² t ⁵ A/i ⁶ A/ m ⁶ t ⁶ A/t ⁶ A/xA/A	6/2/22/ 2/48/4/45
4	Um/U	5/45	37	m ¹ G/02yW/xG/ yW/G	33/6/2/ 7/1
4	Cm/C	4/51	38	ψ/U	17/1
4	Gm/G	2/41	38	m ⁵ C/xC/C	10/1/26
6	m ² G/G	20/36	39	m ⁷ ψ/ψm/ Um/ψ/U	2/6/ 1/79/2
7	m ² G/G	1/69	39	Gm/G	4/32
9	m ¹ A/A	1/69	40	ψ/U	6/1
9	m ¹ G/xG/G	56/1/43	40	m ⁵ C/C	3/139
10	m ² G/G	103/70	44	Um/xU/U	20/2/18
12	ac ⁴ C/C	32/21	e11	ψ/U	2/4
13	ψ/U	47/8	e12	ψ/U	6/11
13	Cm/C	1/83	e14	ψ/U	2/6
14	m ¹ A/xA/A	9/1/165	e2	m ³ C/C	7/7
16	D/U	123/25	46	m ⁷ G/G	86/39
17	D/U	39/0	47	D/xU/U	83/1/16
18	Gm/G	40/138	48	D/U	1/25
20	acp ³ U/D/U	4/118/11	48	m ⁵ C/xC/C	95/1/46
20a	acp ³ U/D/ψ/xU/U	6/64/2/2/2	49	xA/A	1/19
20b	D/ψ/U	8/6/2	49	m ⁵ C/xC/C	64/1/13
25	ψ/U	1/28	50	ψ/U	4/45
26	ψ/U	1/24	50	m ⁵ C/C	15/71
26	m ²² G/m ² G/xG/G	90/16/2/8	54	m ⁵ Um/m ⁵ U/ψ/U	16/111/11/17
27	ψ/U	70/10	55	ψ/U	166/12
27	m ²² G/G	3/14	58	m ⁷ A/xA/A	151/1/26
28	ψ/U	37/38	64	Ar(p)/A	2/43
30	ψ/U	1/4	64	Gr(p)/xG/G	1/1/87
31	ψ/U	4/2	65	ψ/U	1/32
32	ψm/Um/ψ/U	2/6/24/8	67	ψ/U	2/63
32	Cm/m ³ C/xC/C	40/16/2/80	67	m ² G/G	2/64
34	I/A	32/0	68	ψ/U	1/37
34	s ² U/Um/mchm ⁵ U/ ncm ⁵ U/cnm ⁵ Um/ mcm ⁵ s ² U/mcm ⁵ U/ ψ/xU/U	1/3/1/ 3/1/ 7/4/ 1/11/5	72	ψ/U	1/16
34	Cm/t ⁵ Cm/m ⁵ C/xC/C	8/1/1/2/44	72	m ⁵ C/C	5/132
34	QtRNA/manQtRNA/ galQtRNA/GmG	5/4/ 3/17/20			

FIGURE 2B
SUBSTITUTE SHEET (RULE 26)



WELLS

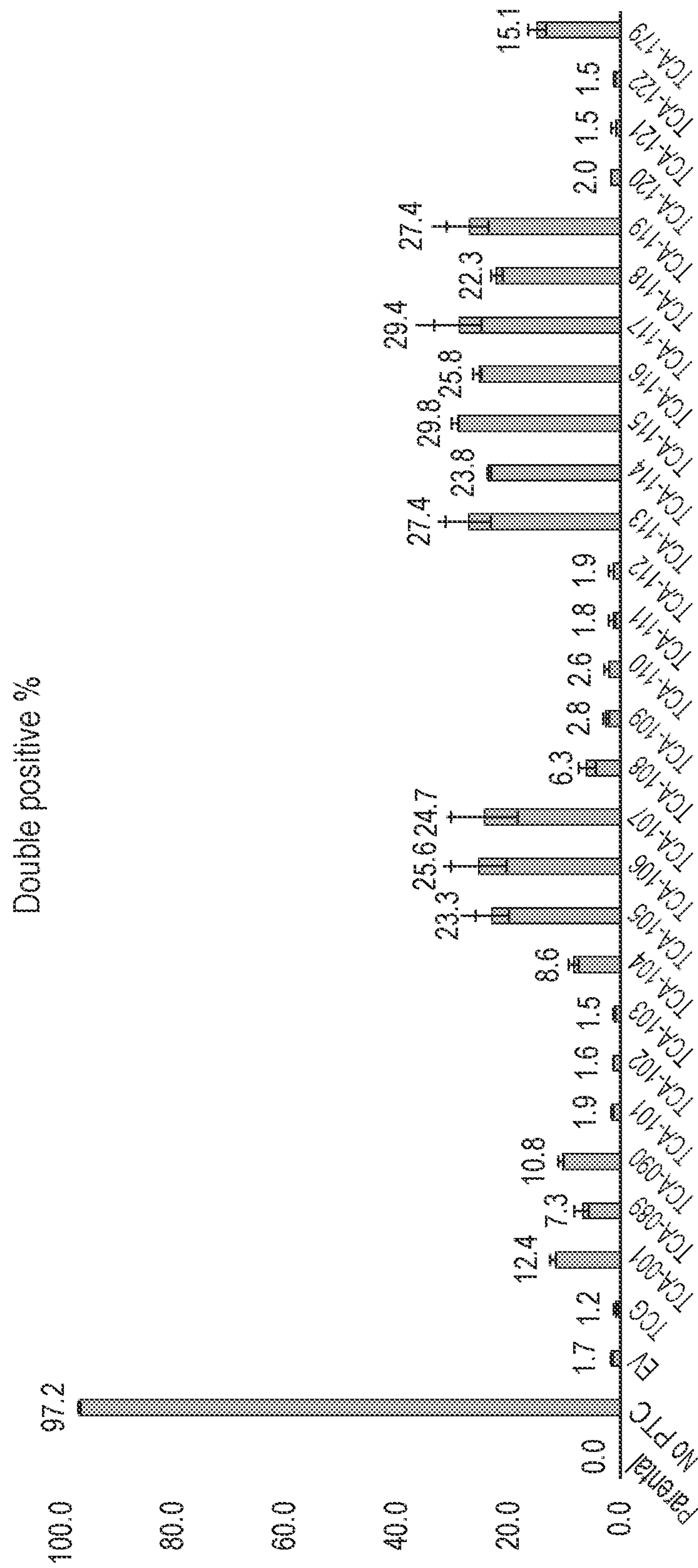


FIGURE 4

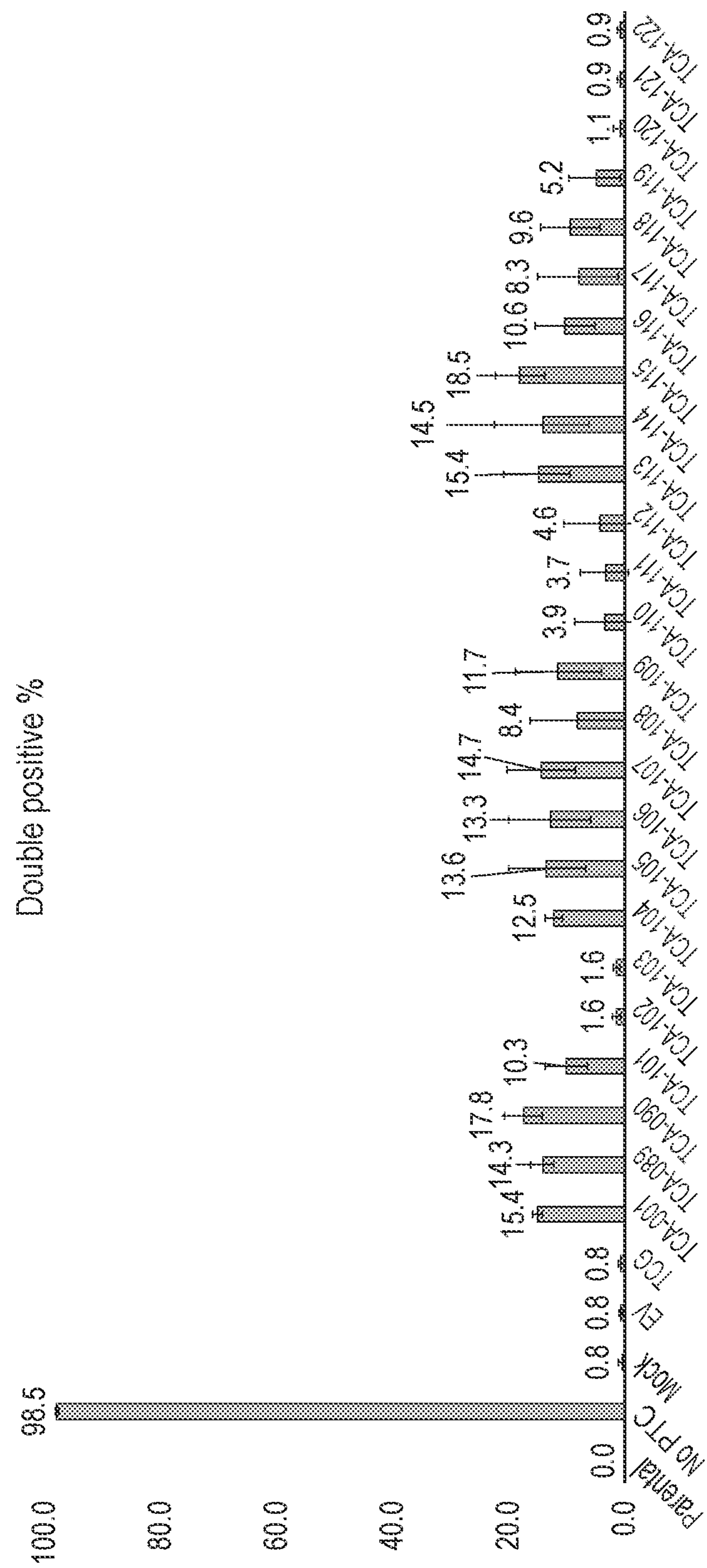


FIGURE 5

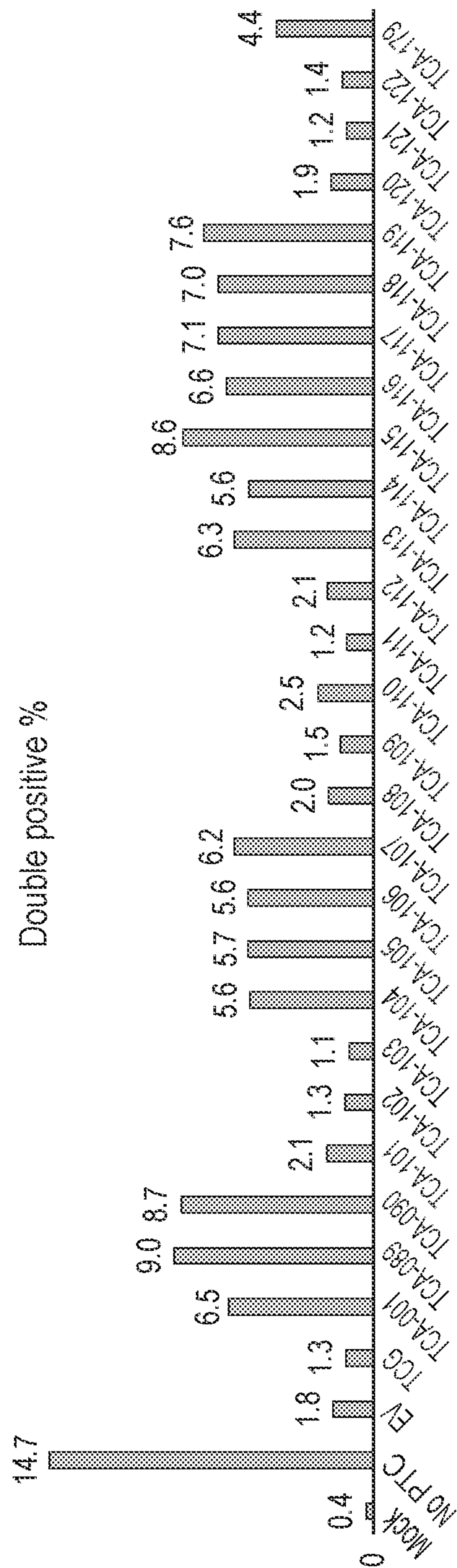


FIGURE 6

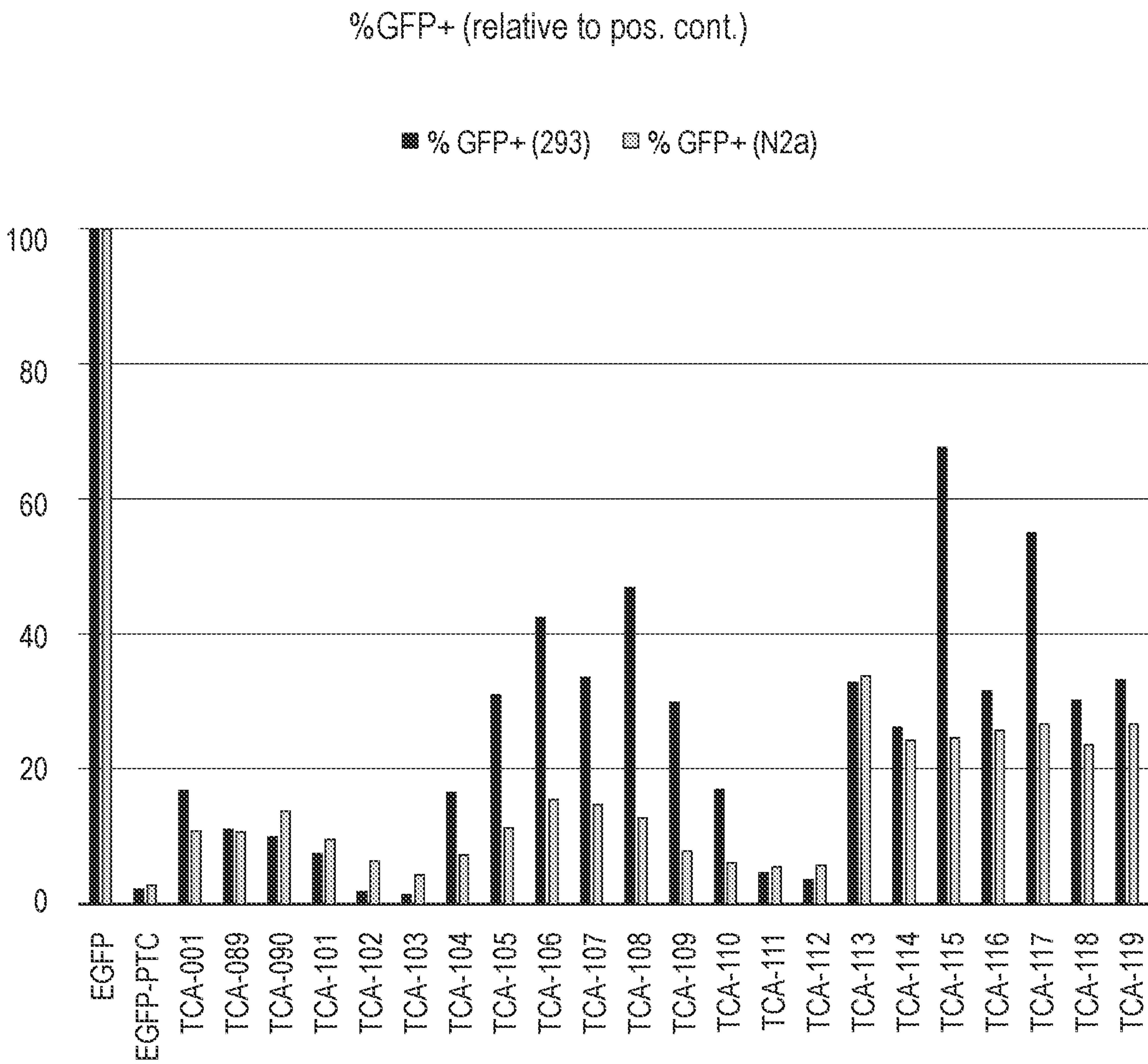


FIGURE 7

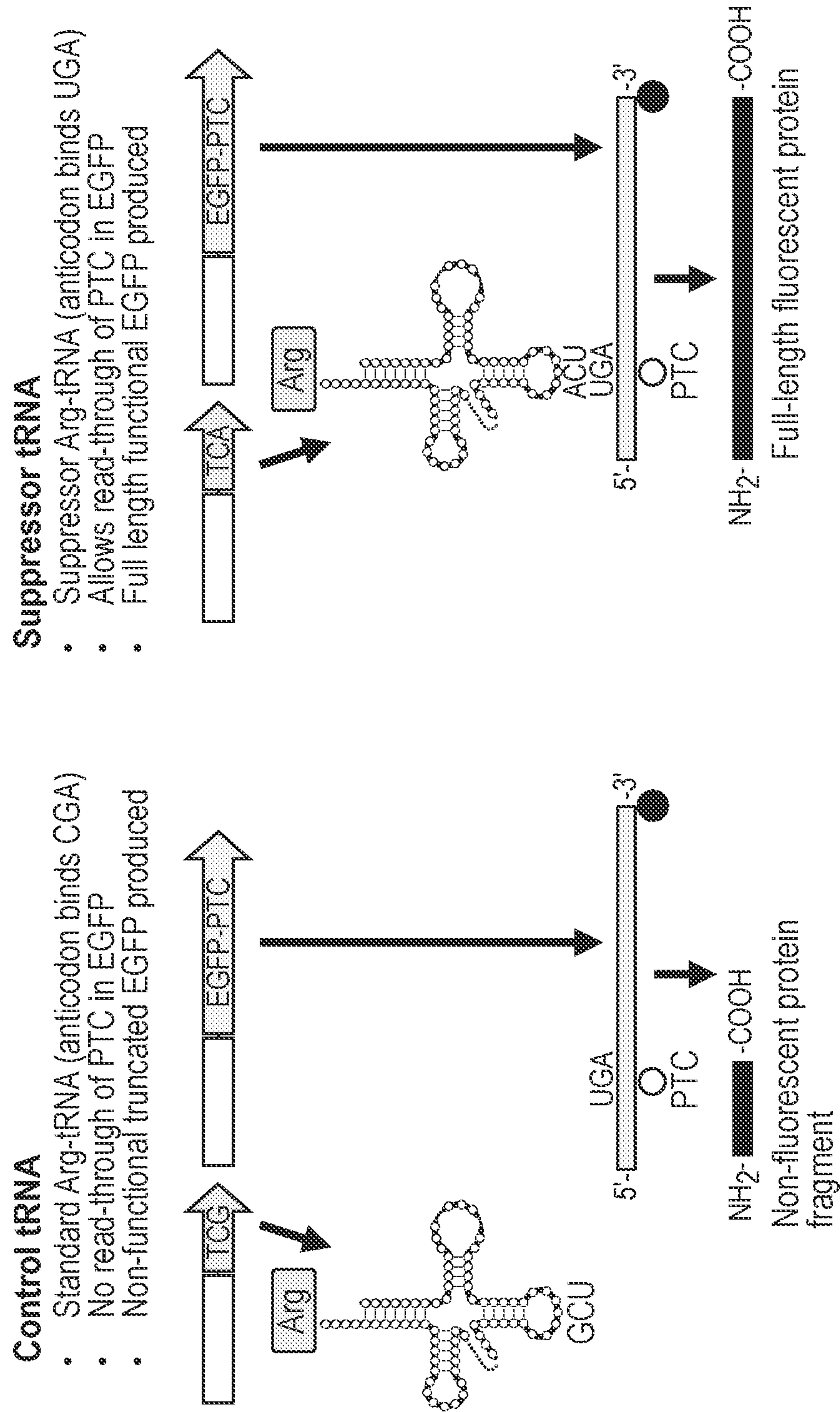


FIGURE 8A

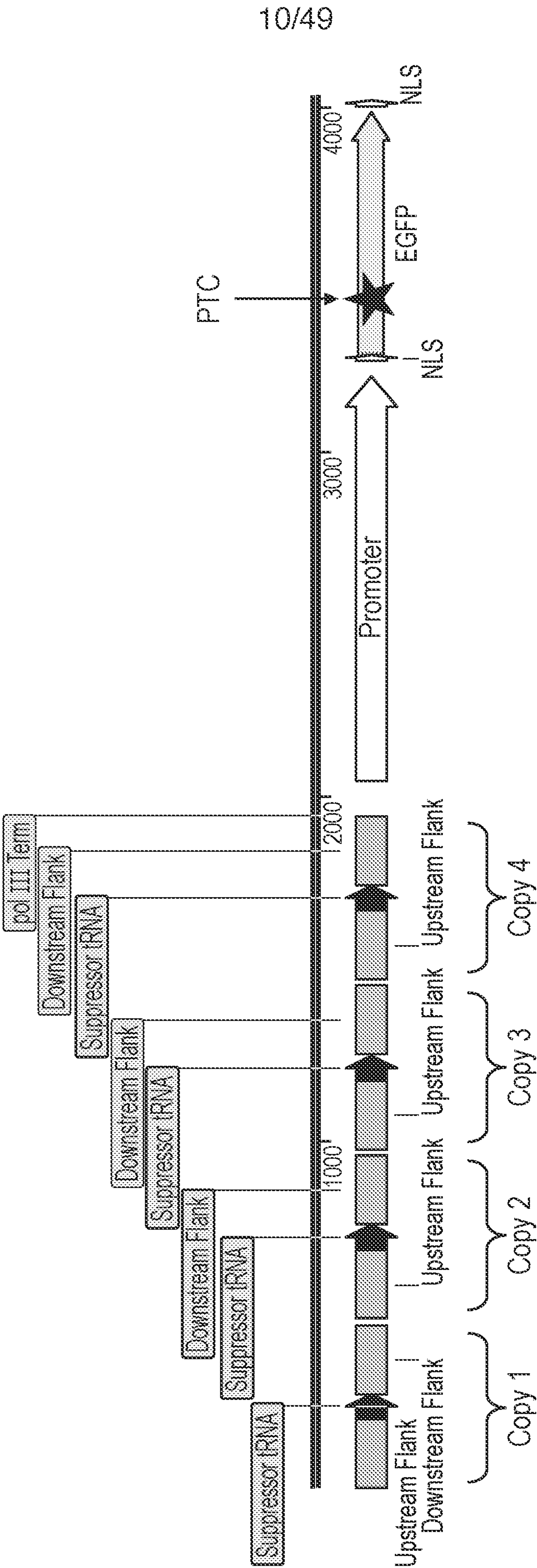


FIGURE 8B

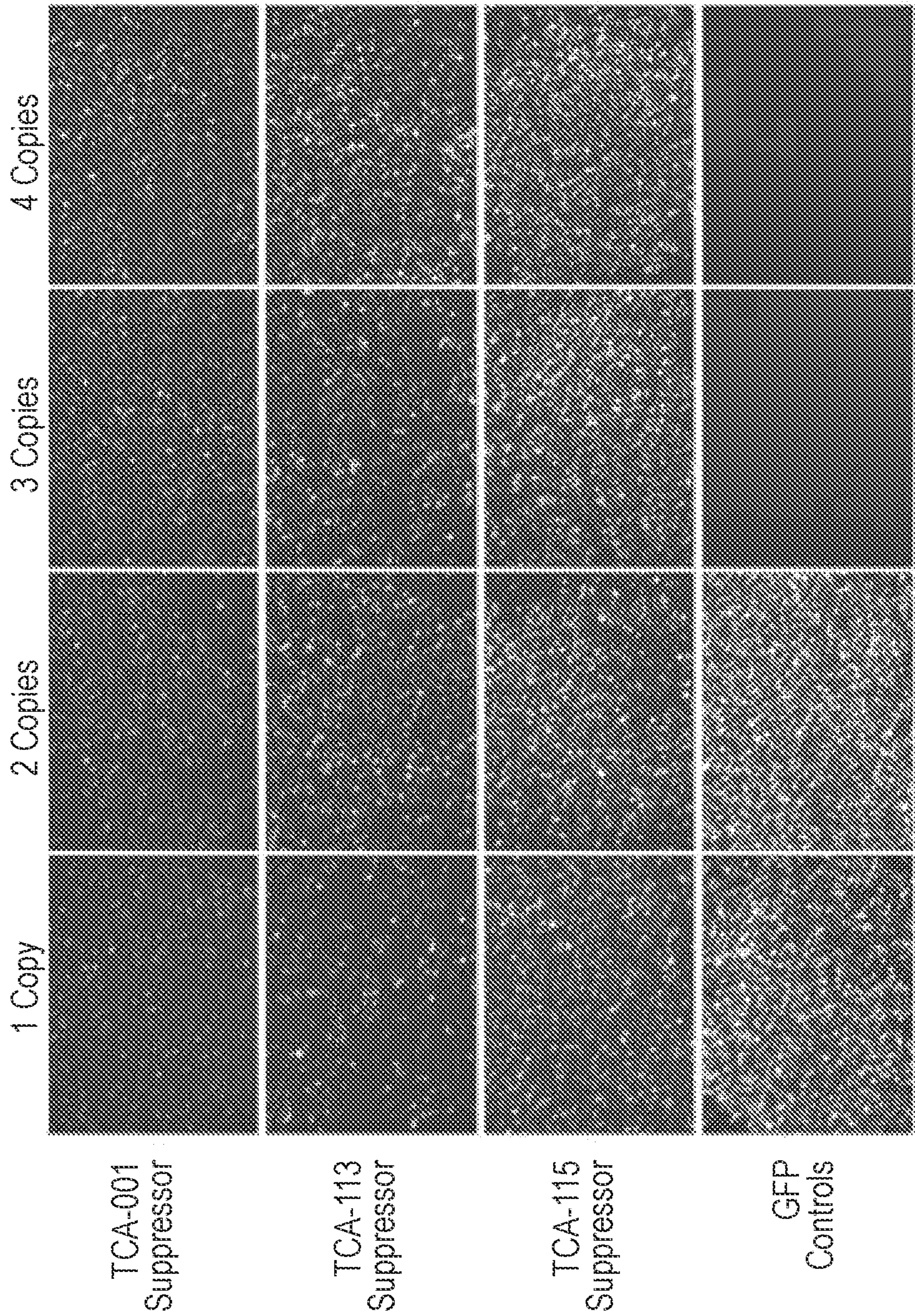


FIGURE 9

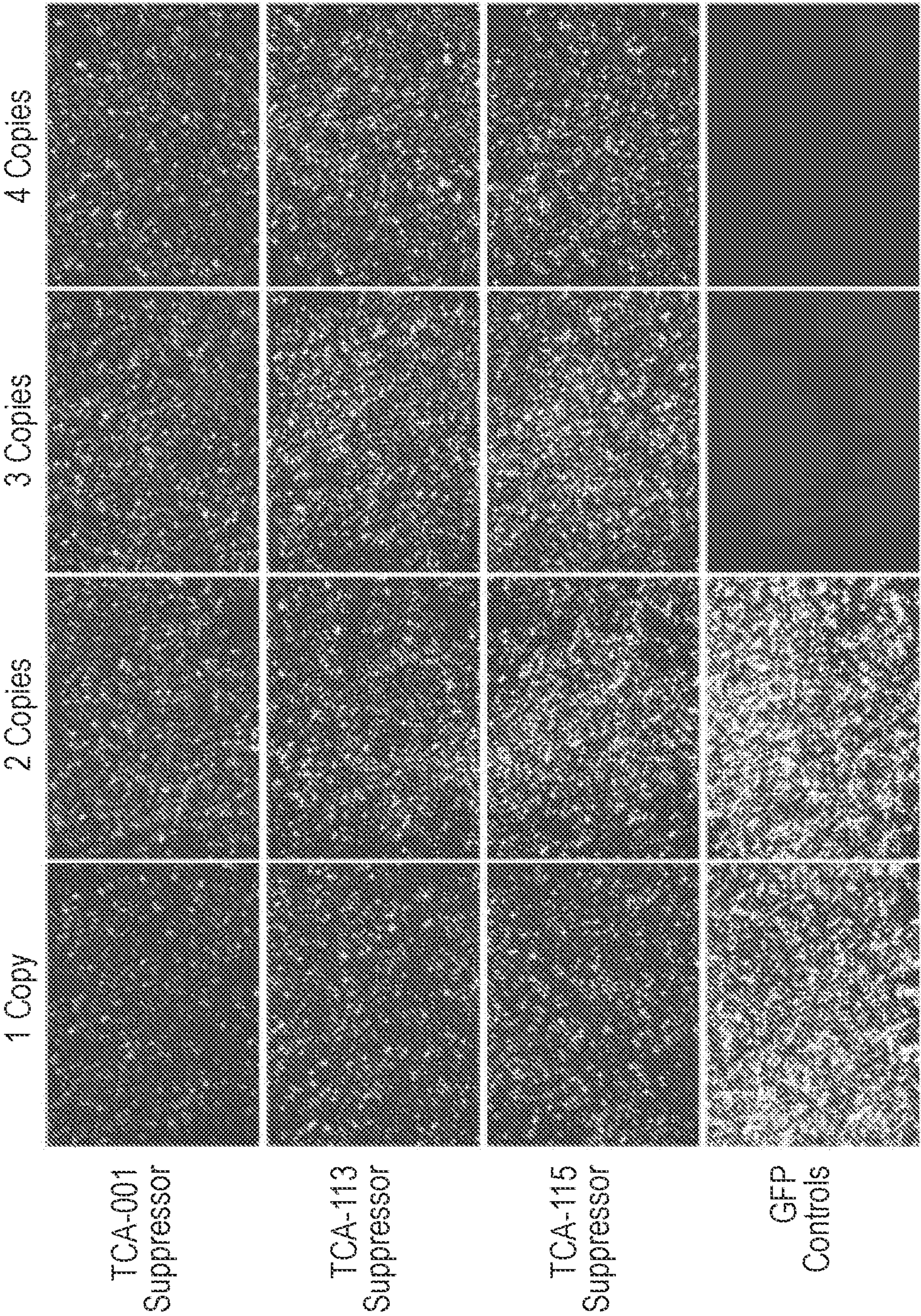


FIGURE 10

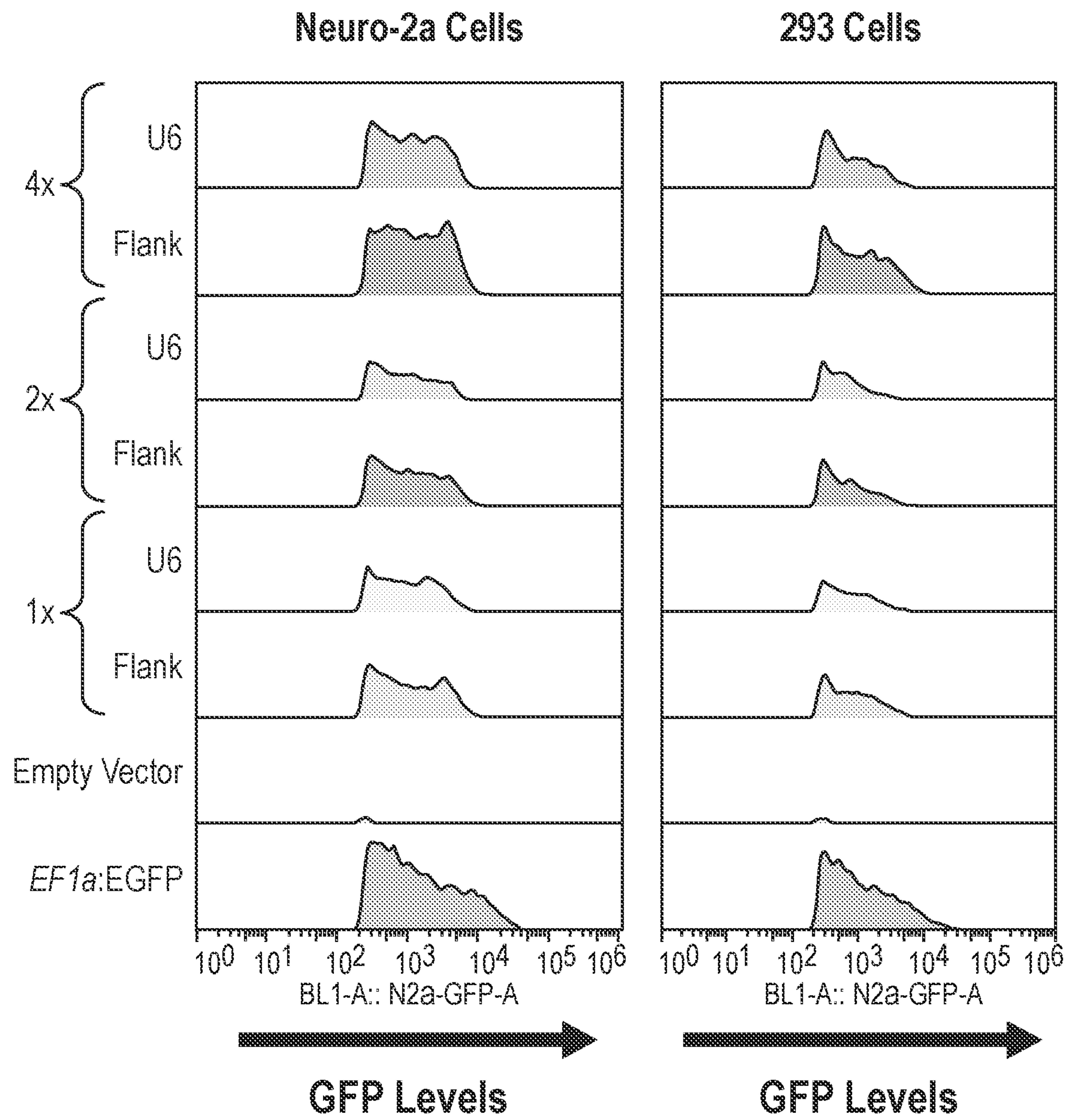
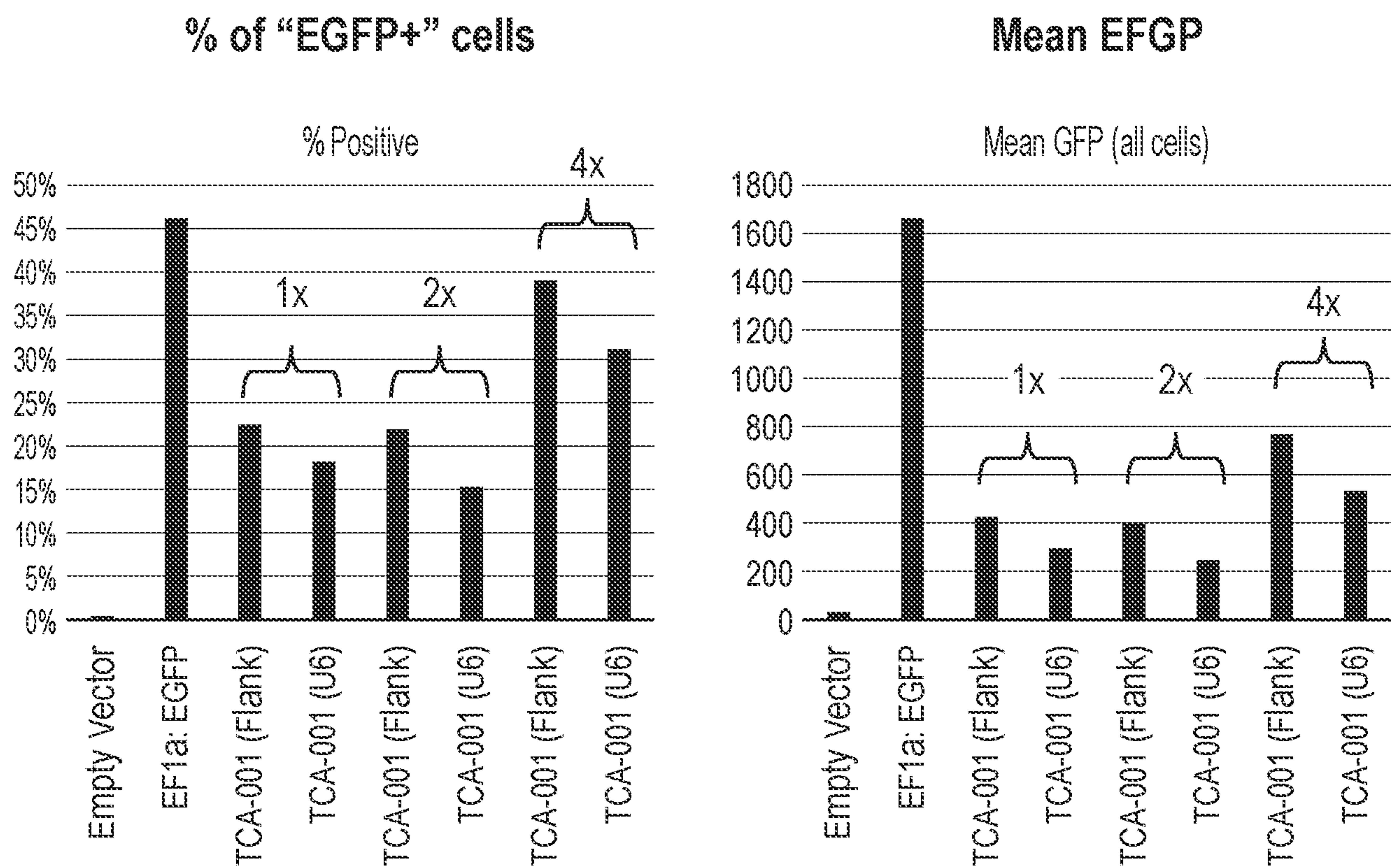


FIGURE 11



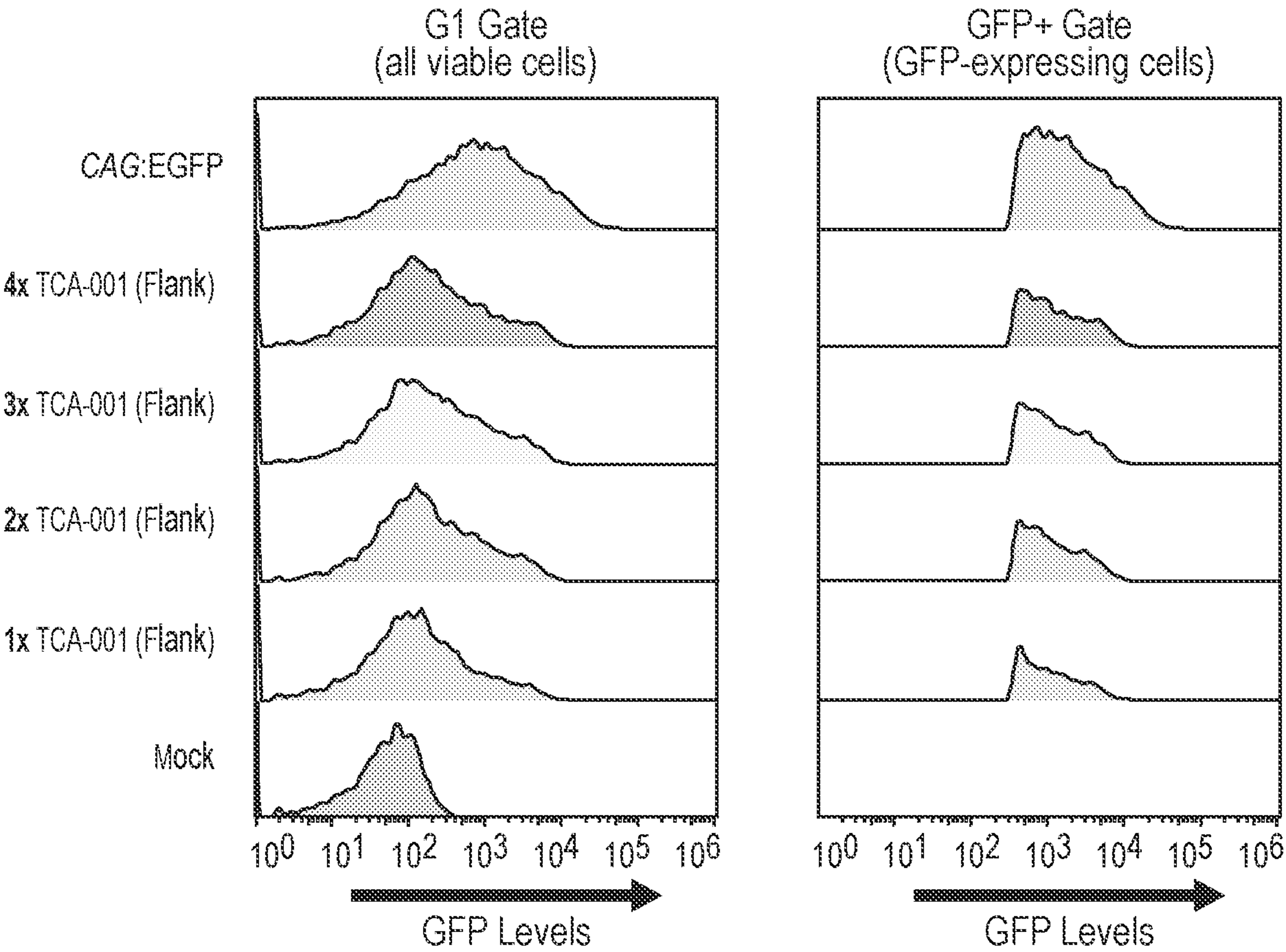


FIGURE 13

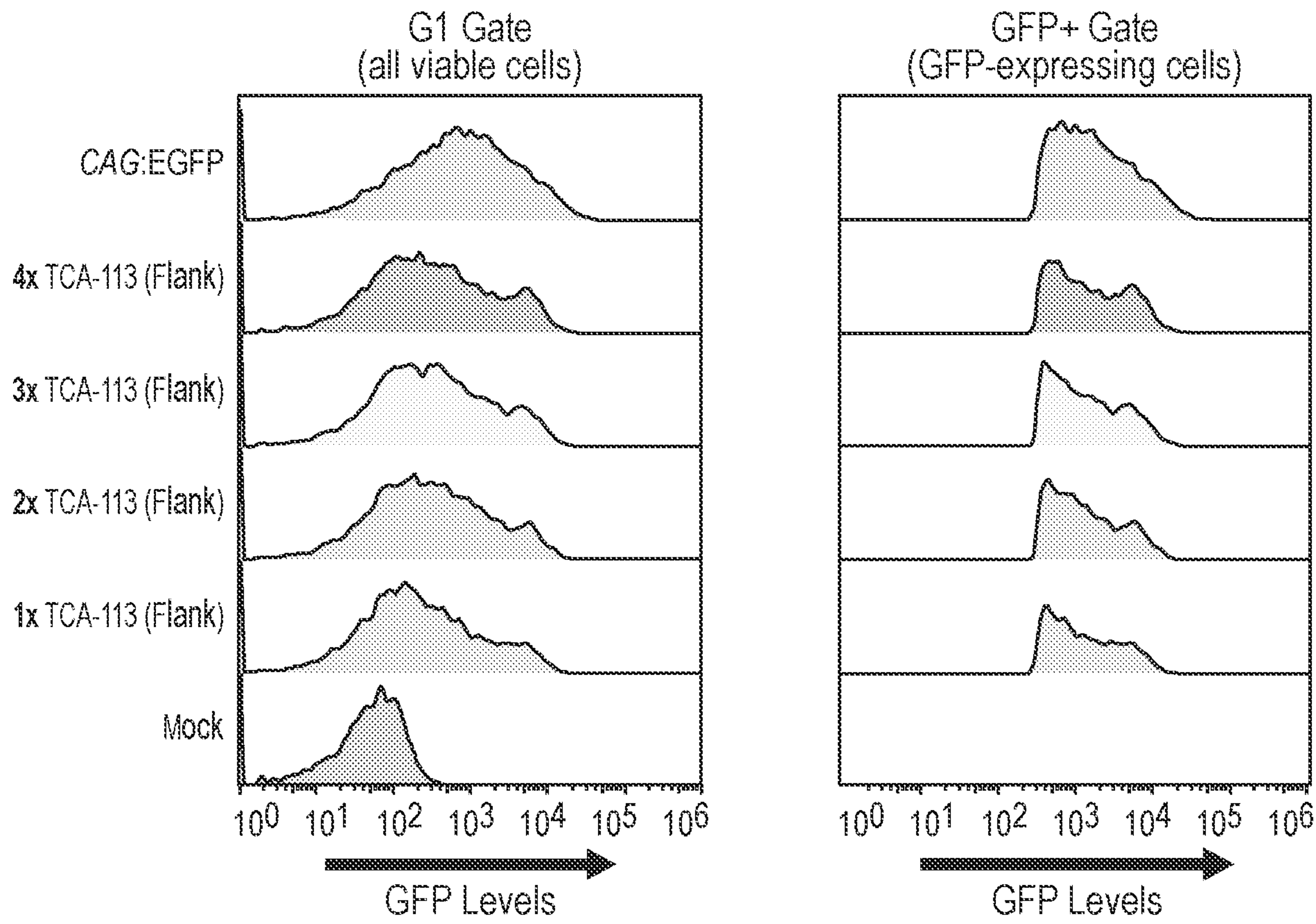


FIGURE 14

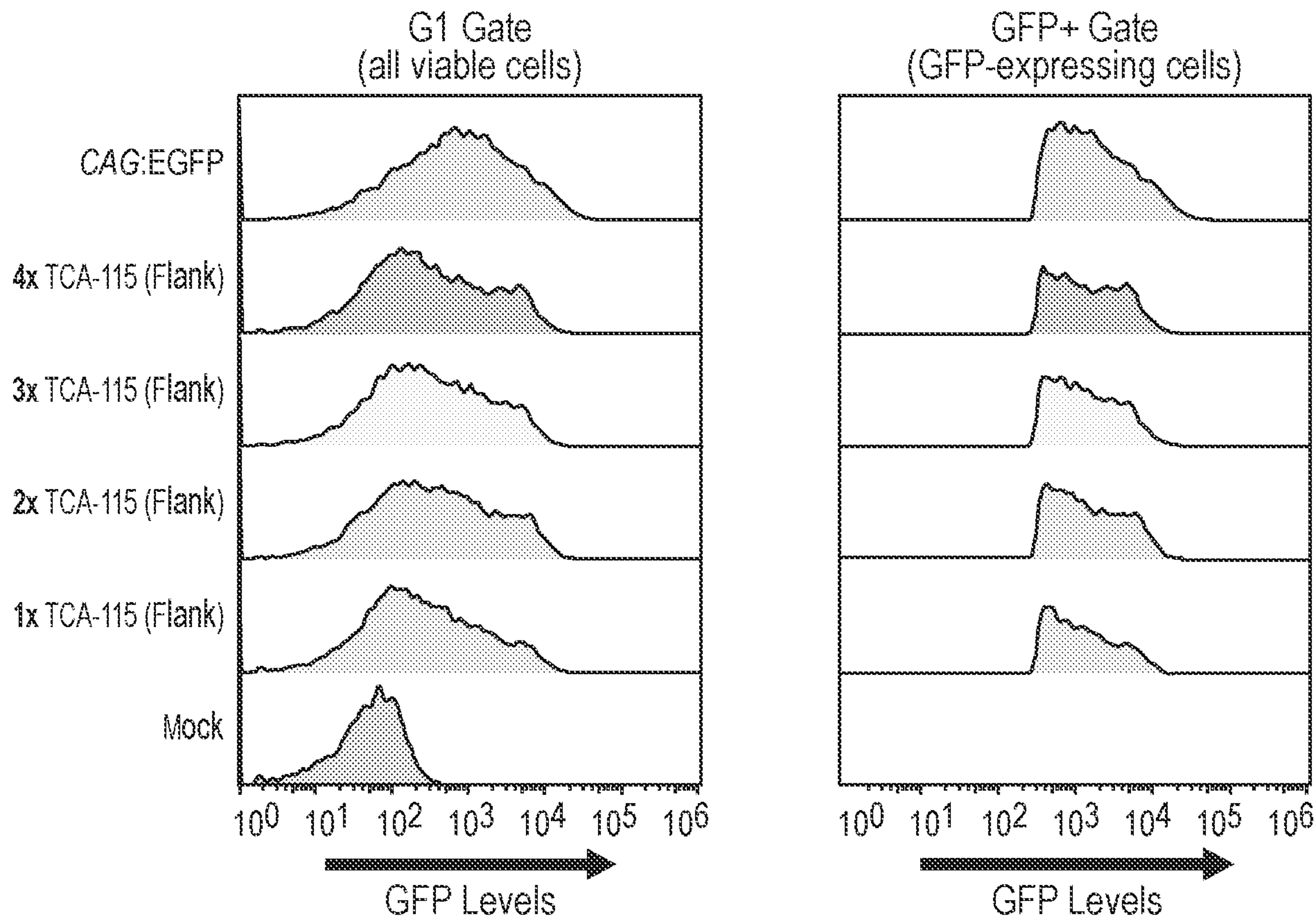


FIGURE 15

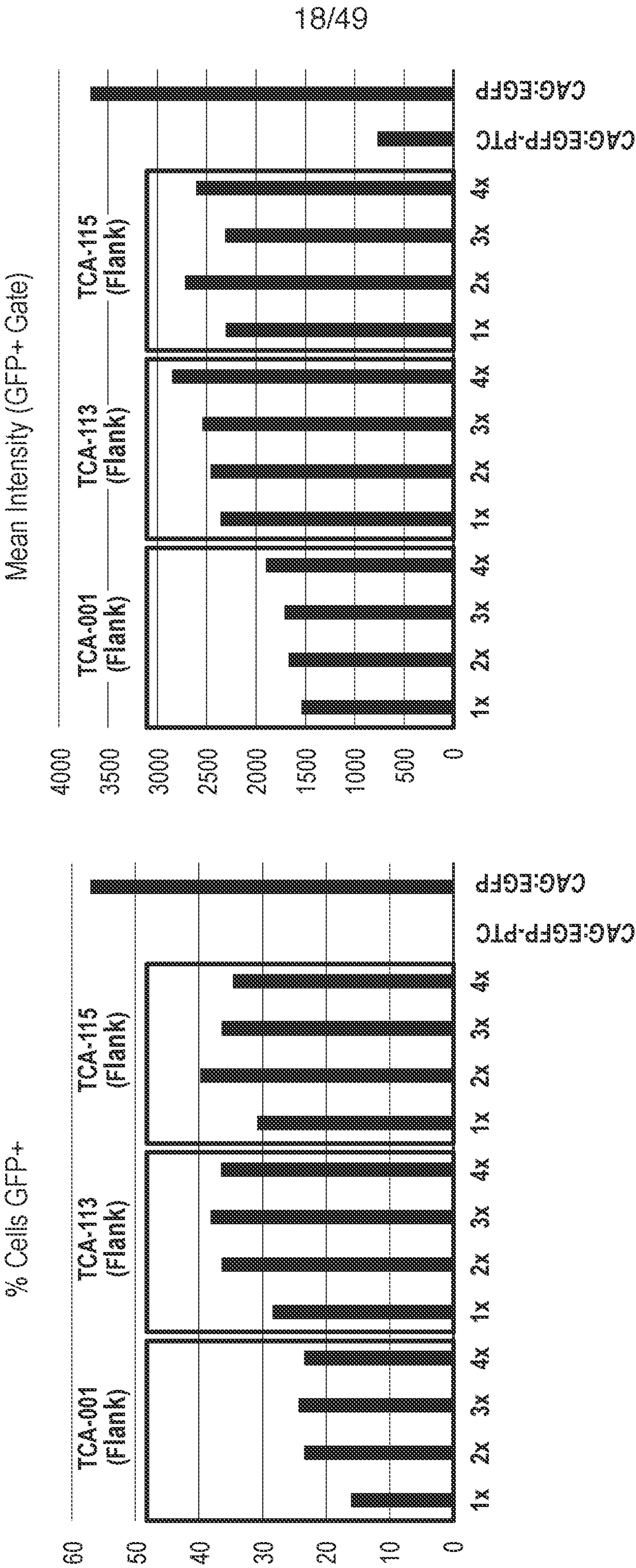


FIGURE 16

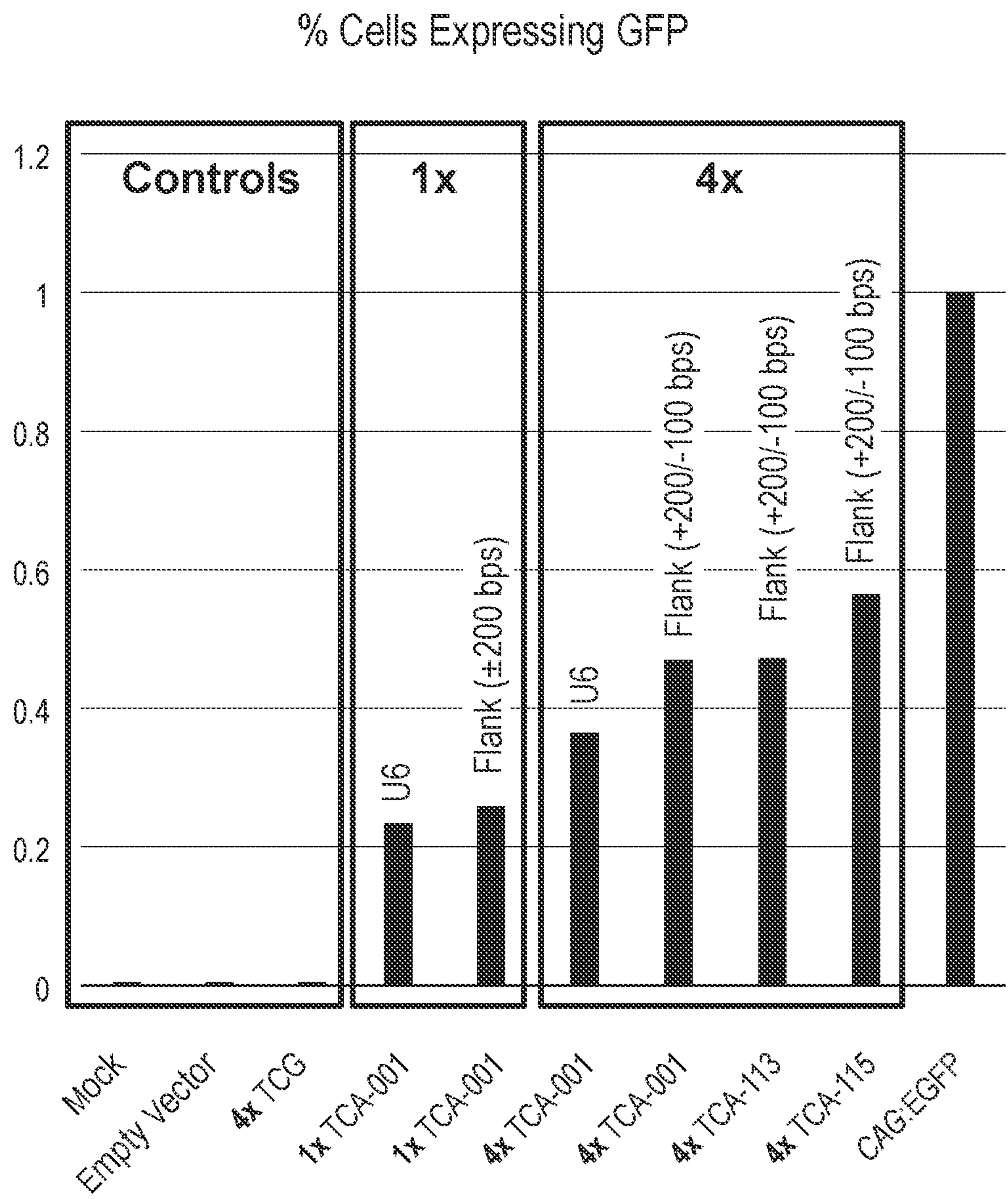


FIGURE 17

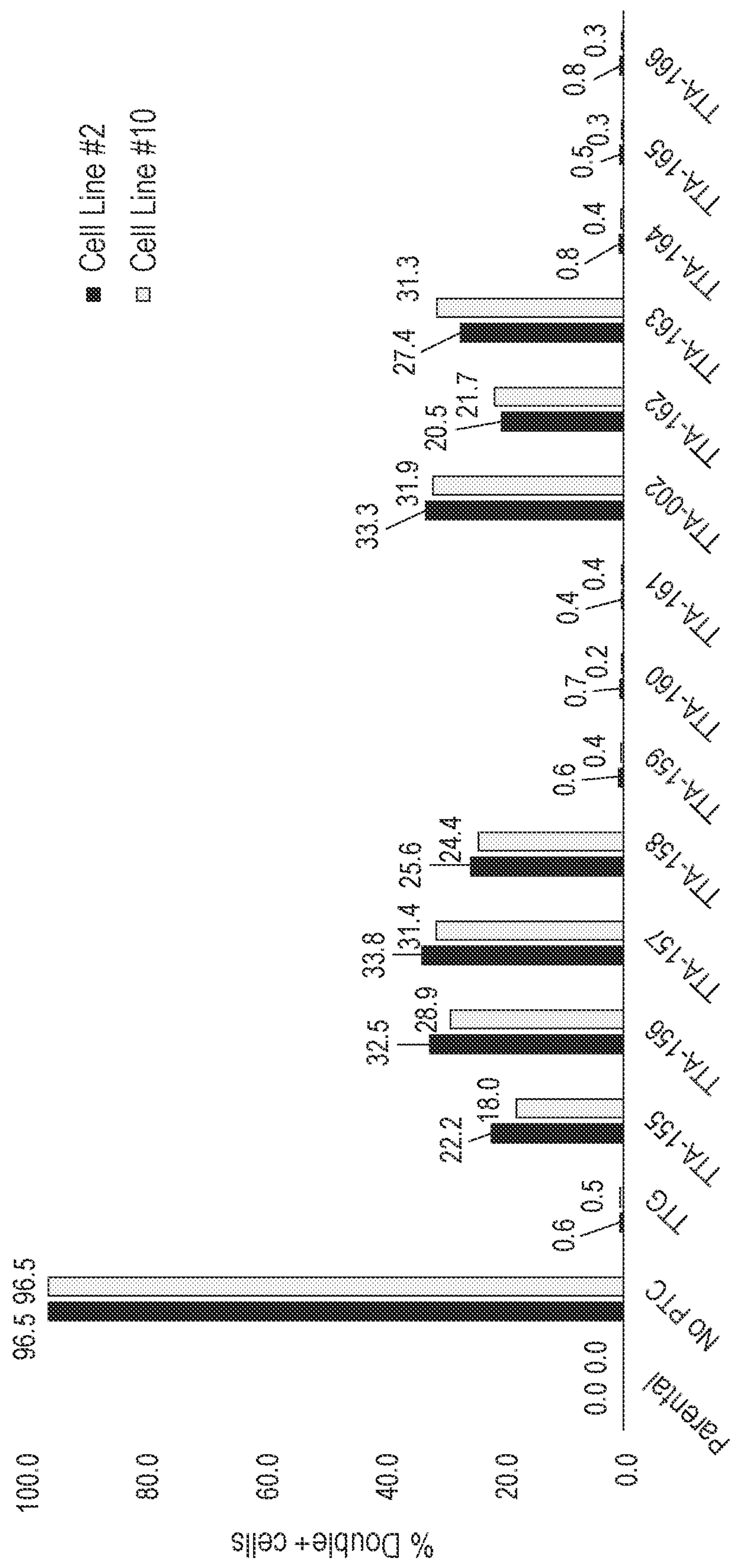


FIGURE 18

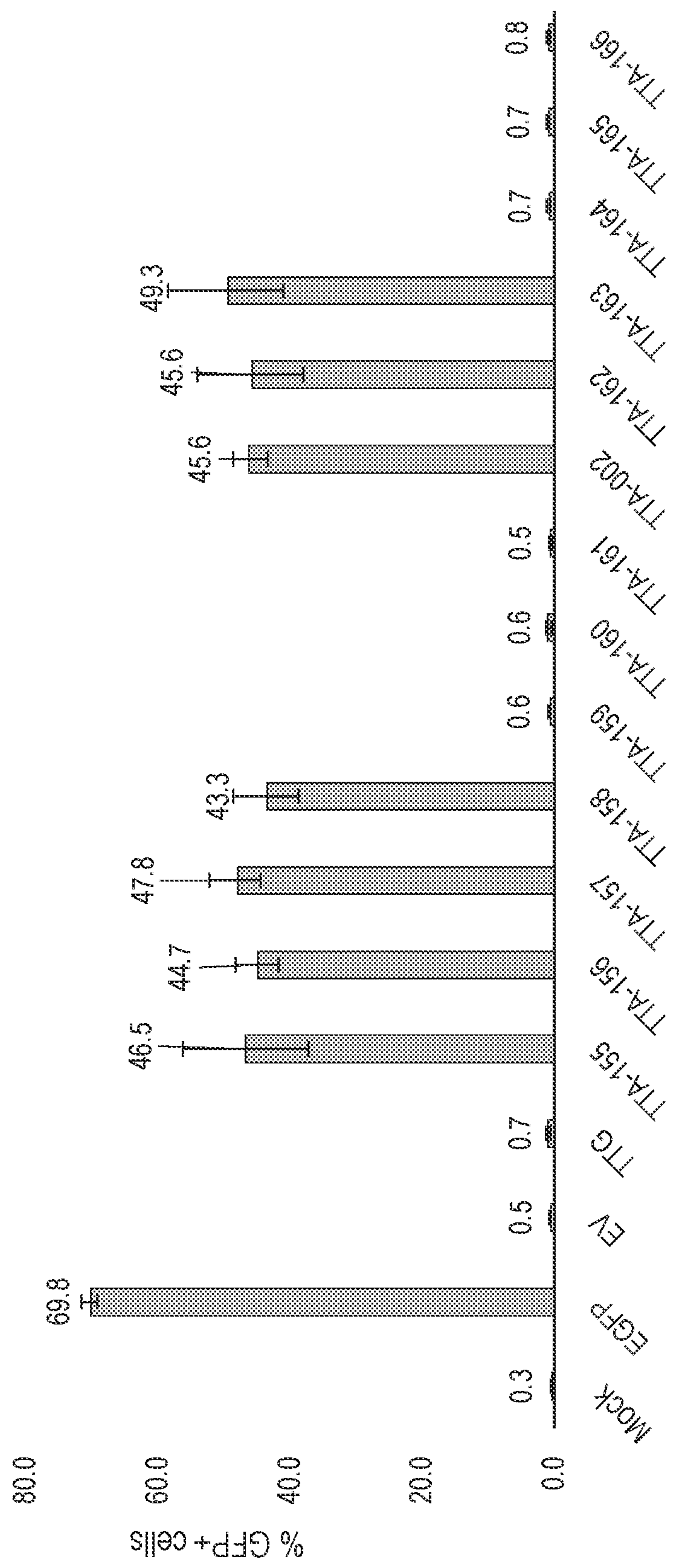


FIGURE 19

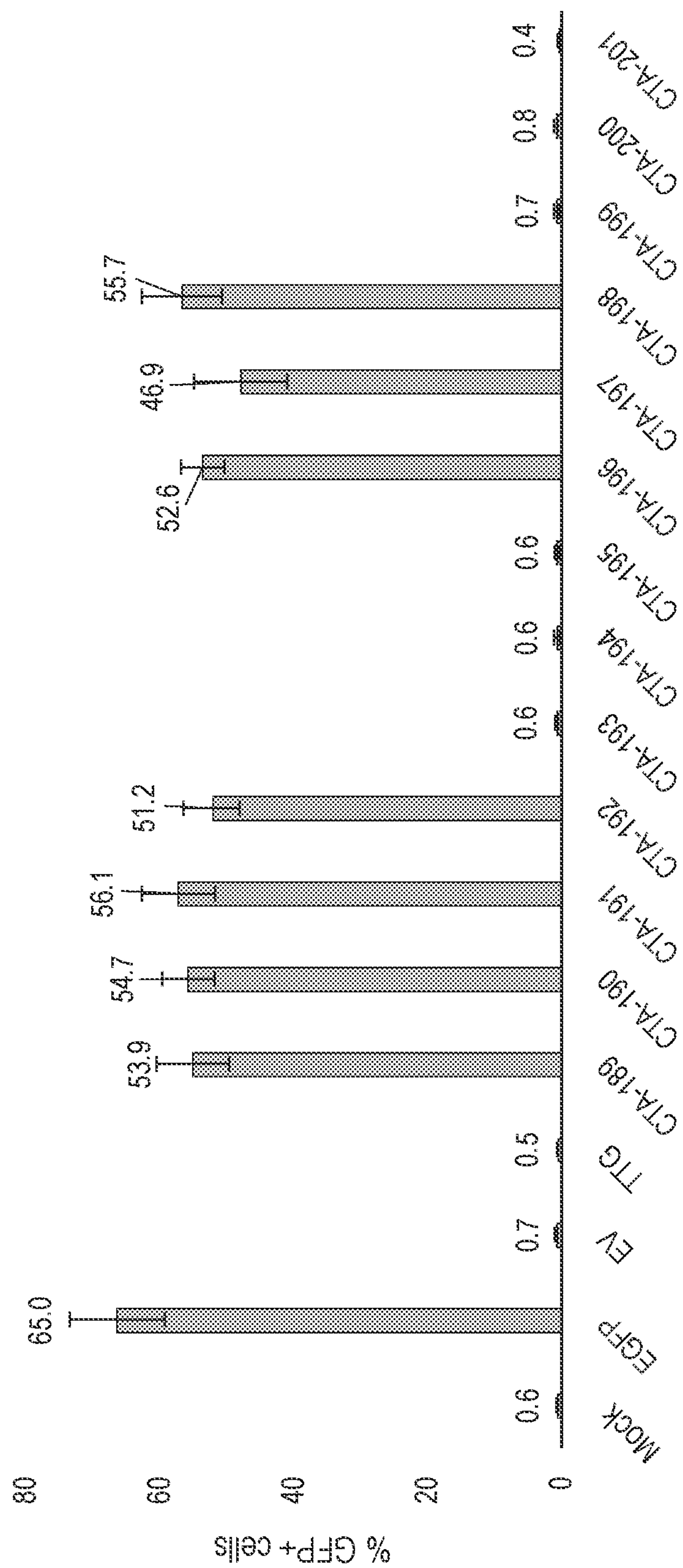


FIGURE 20

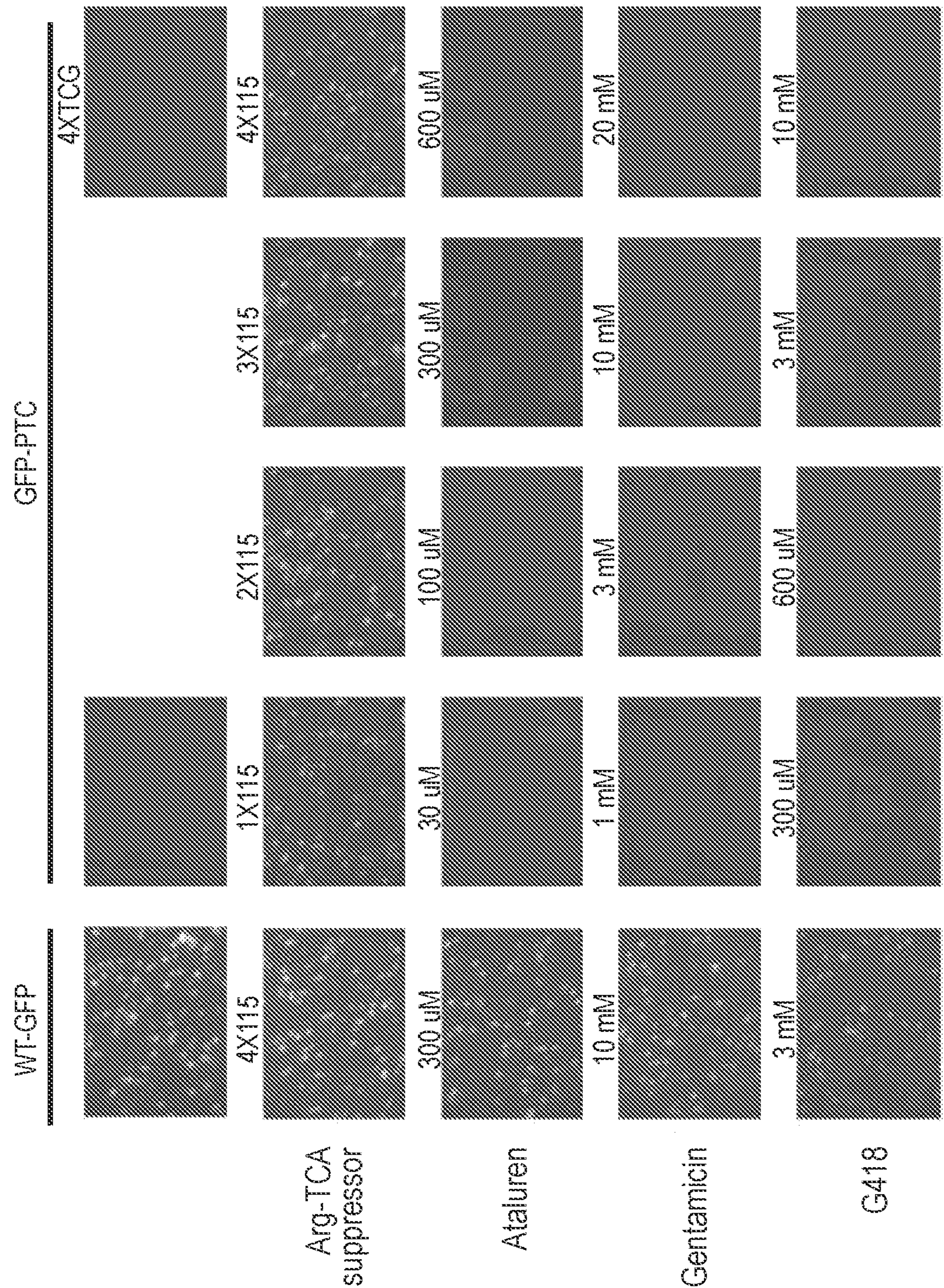


FIGURE 21

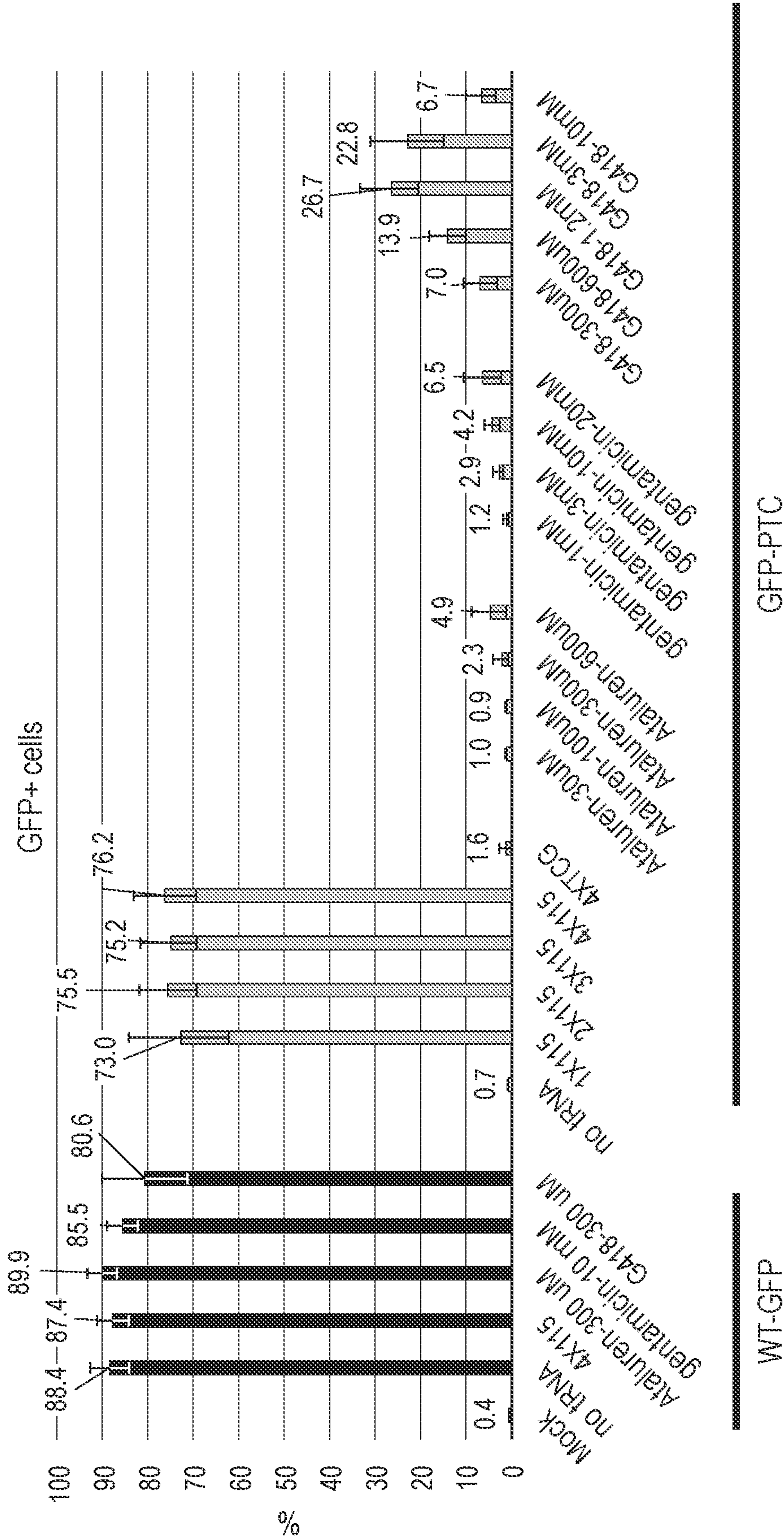


FIGURE 22

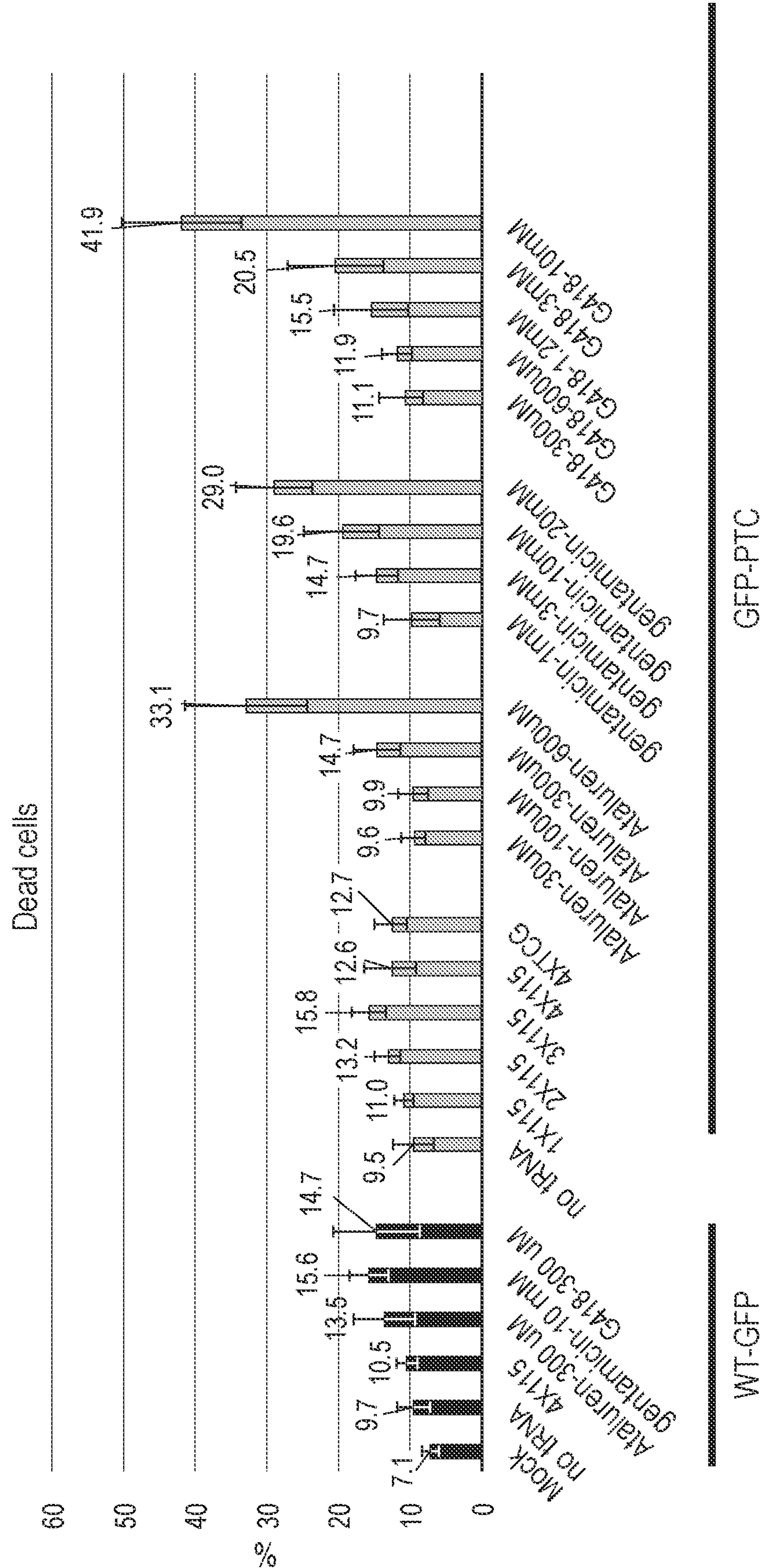


FIGURE 23

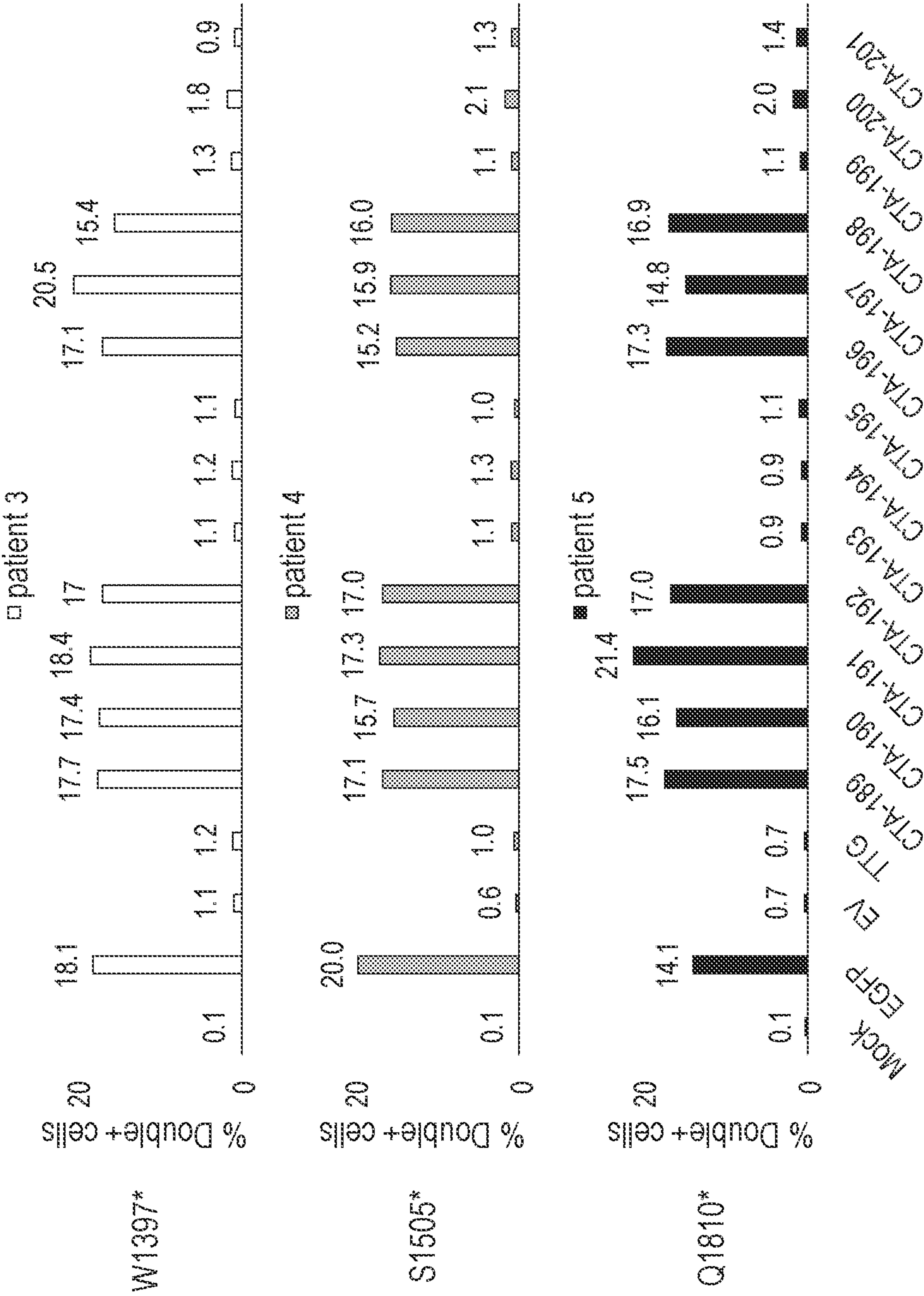


FIGURE 24

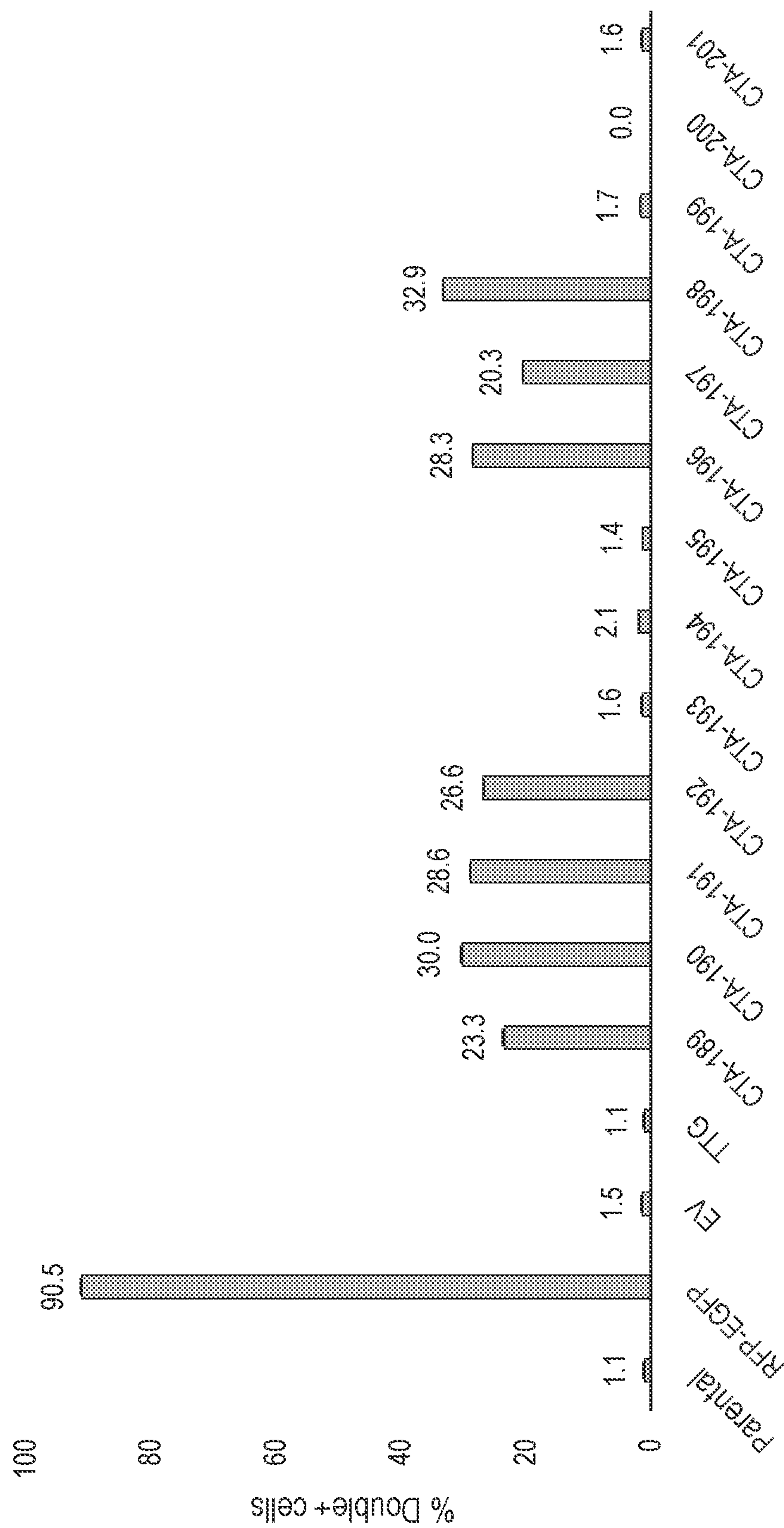


FIGURE 25

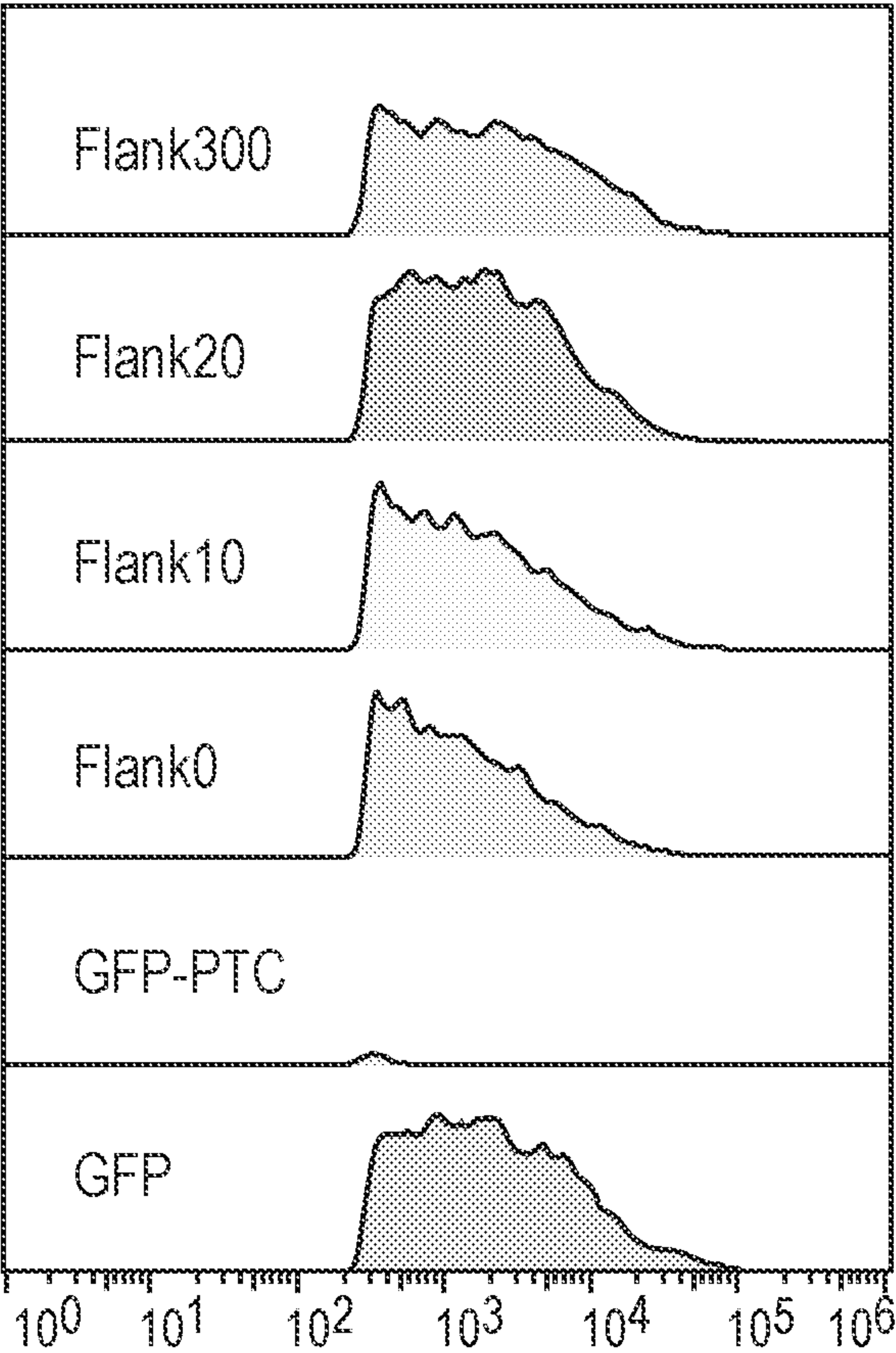


FIGURE 26A

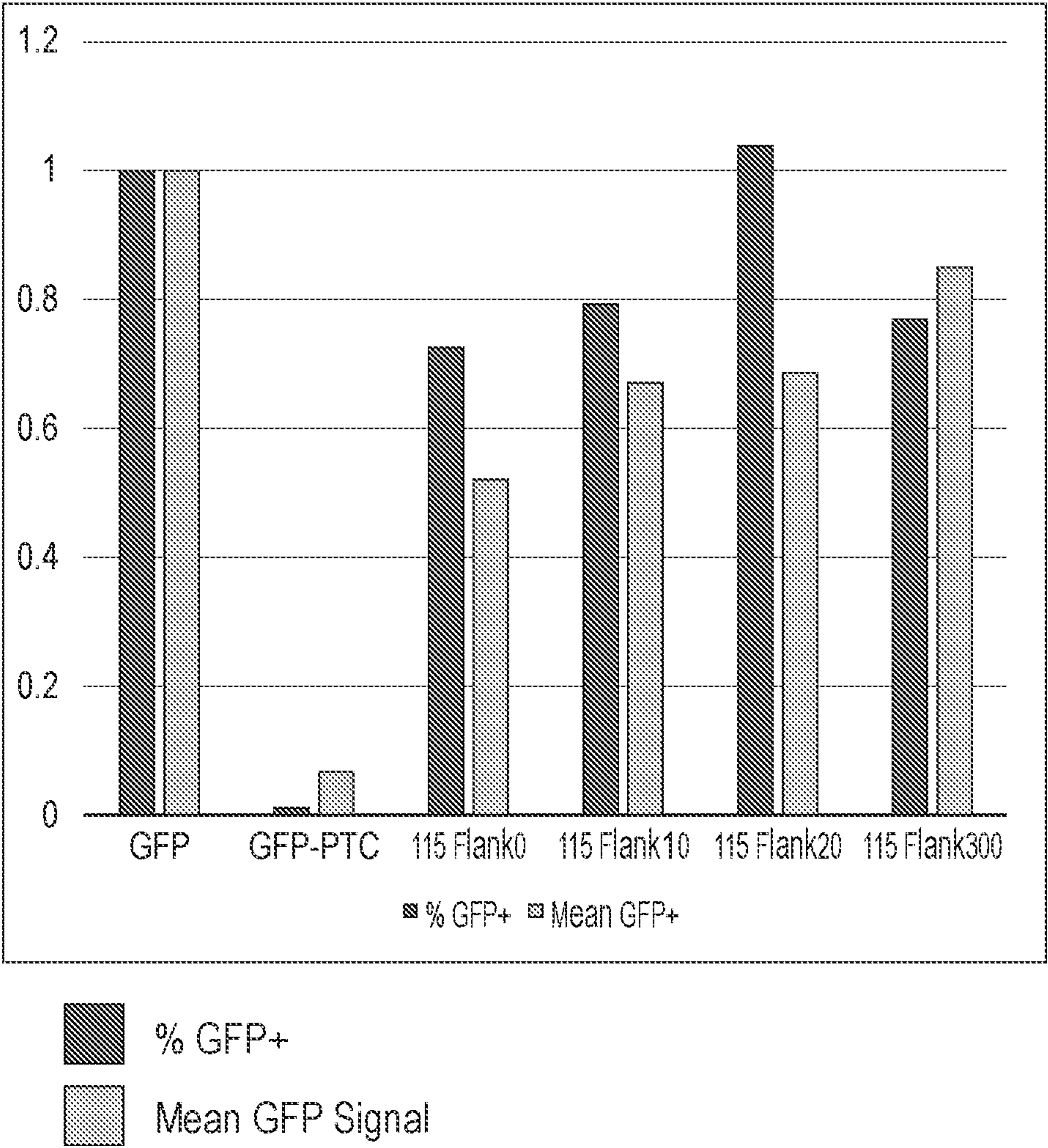


FIGURE 26B

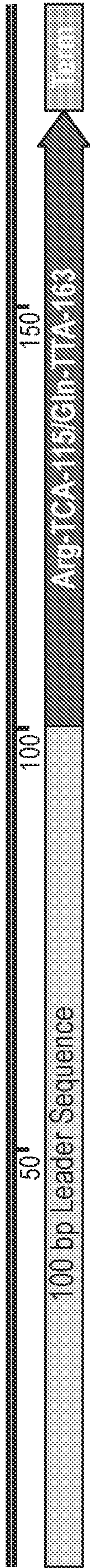


FIGURE 27

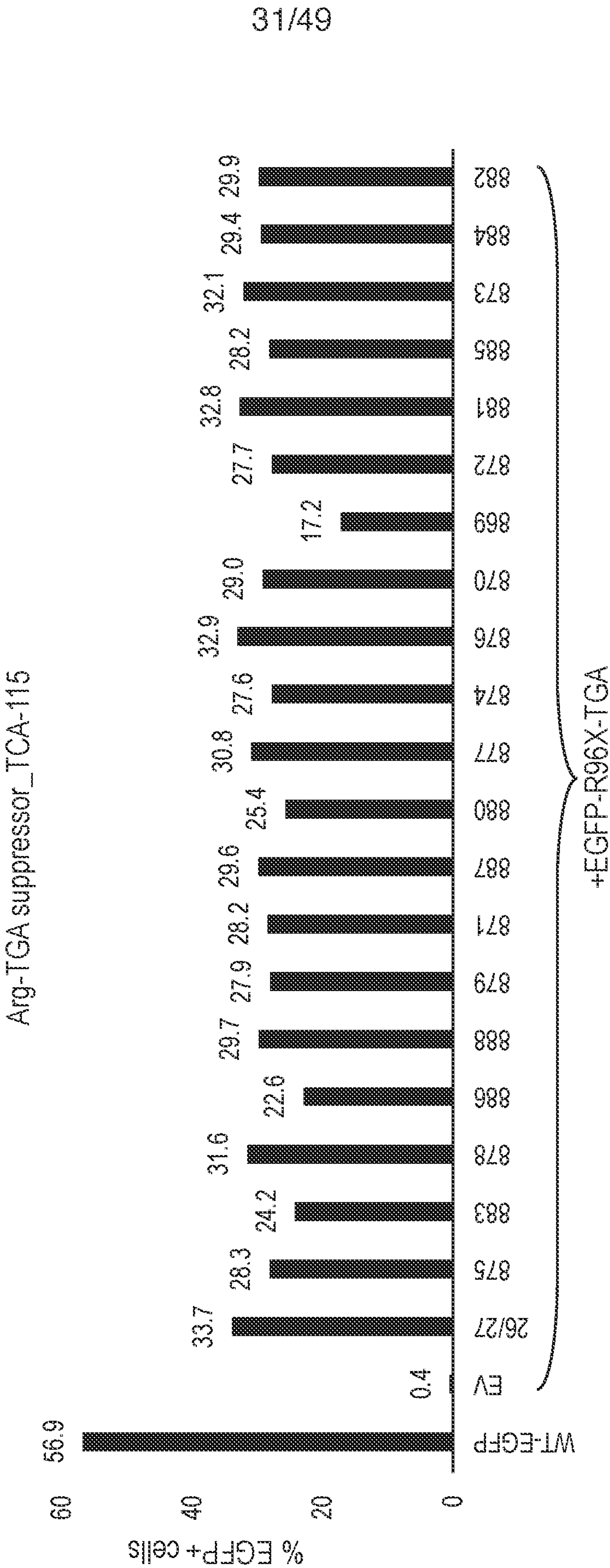


FIGURE 28

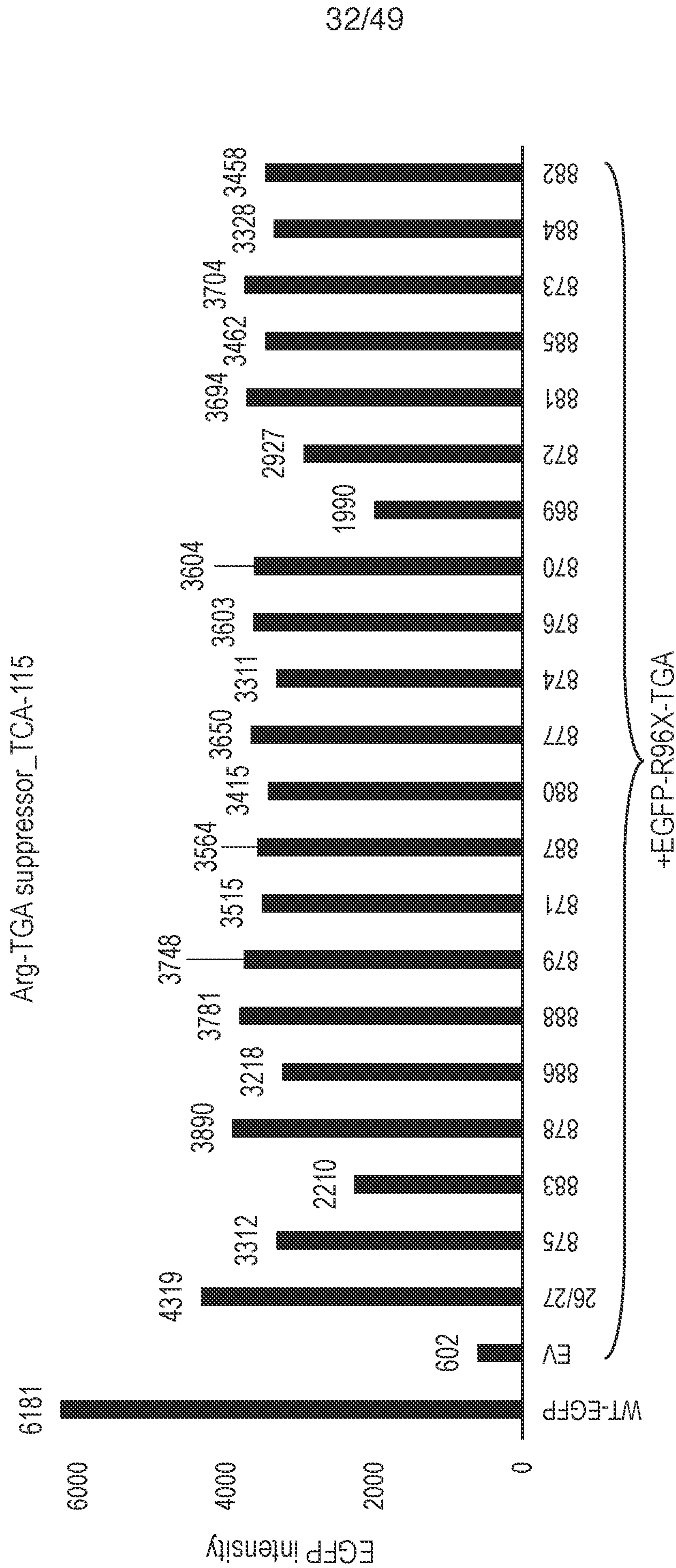


FIGURE 29

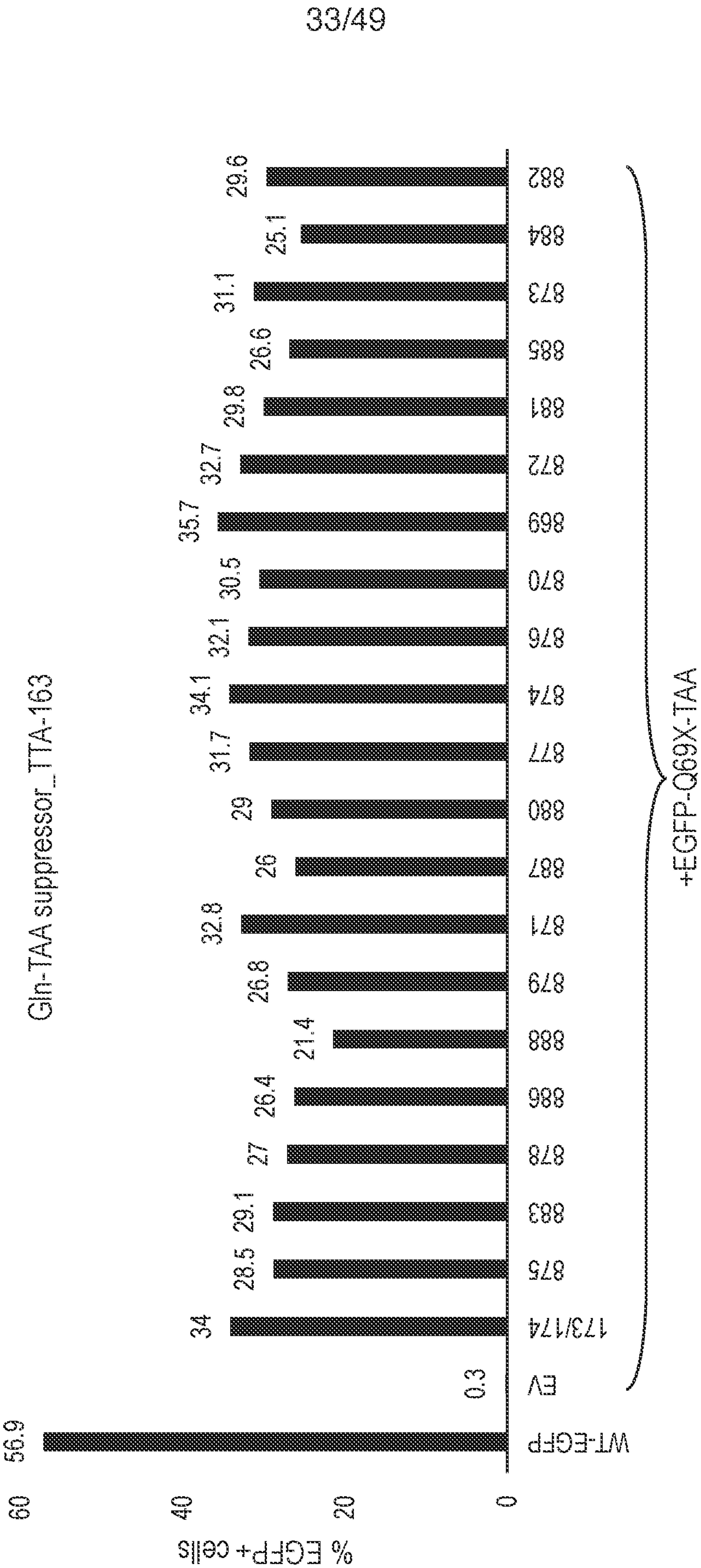


FIGURE 30

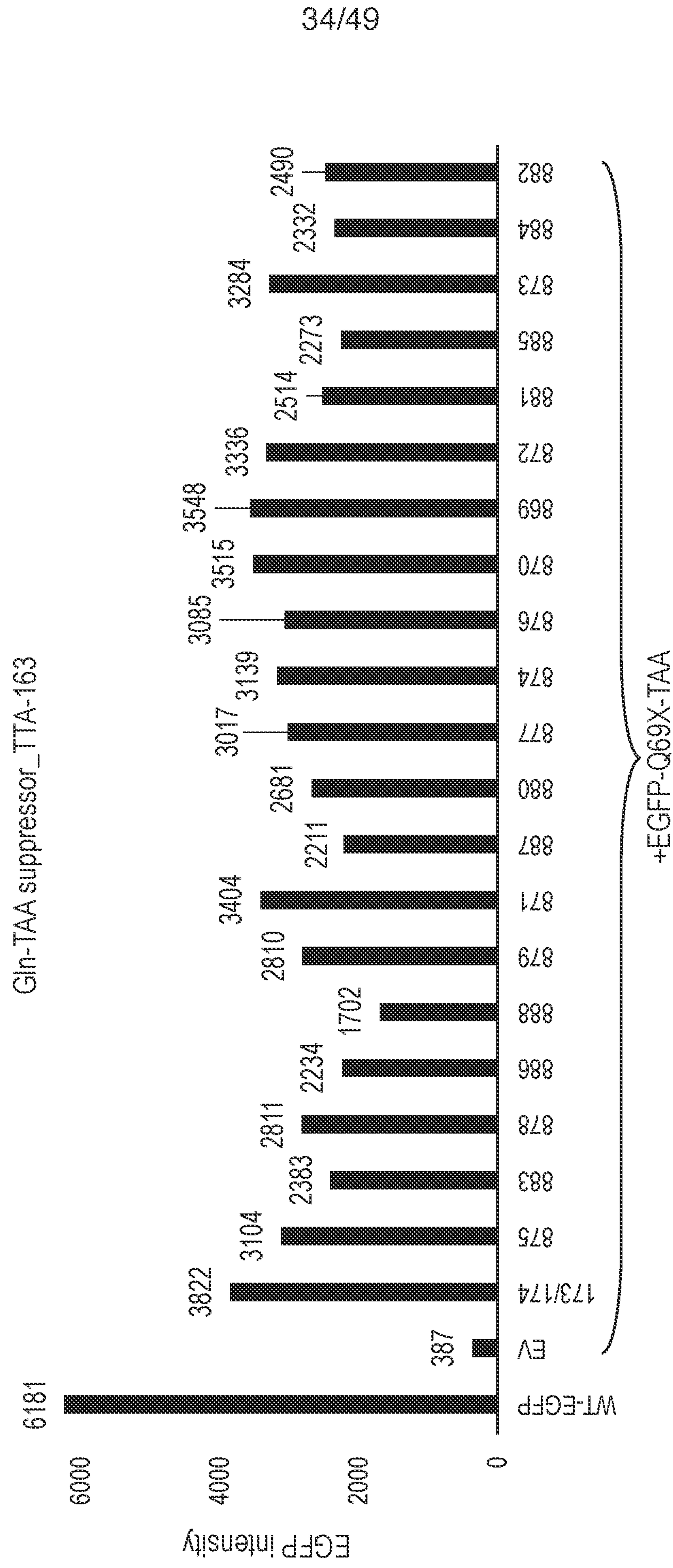


FIGURE 31

SEQ ID NO	Leader Name	GlnTTA (%GFP+)	GlnTTA (Mean Intensity)	ArgTCA (%GFP+)	ArgTCA (Mean Intensity)
869	Ser-TGA-4-1	62.74%	57.40%	30.23%	32.20%
870	Ser-CGA-4-1	53.60%	56.87%	50.97%	58.31%
871	Arg-TCG-5-1	57.64%	55.07%	49.56%	56.87%
872	Ser-GCT-2-1	57.47%	53.97%	48.68%	47.35%
873	Ile-AAT-4-1	54.66%	53.13%	56.41%	59.93%
874	Ala-AGC-4-1	59.93%	50.78%	48.51%	53.57%
875	Arg-TCT-1-1	50.09%	50.22%	49.74%	53.58%
876	Leu-TAA-1-1	56.41%	49.91%	57.82%	58.29%
877	Arg-CCG-2-1	55.71%	48.81%	54.13%	59.05%
878	Ser-GCT-3-1	47.45%	45.48%	55.54%	62.93%
879	Ser-TGA-1-1	47.10%	45.46%	49.03%	60.64%
880	Asn-GTT-1-1	50.97%	43.37%	44.64%	55.25%
881	Arg-TCT-2-1	52.37%	40.67%	57.64%	59.76%
882	Asn-GTT-3-1	52.02%	40.28%	52.55%	55.95%
883	Tyr-GTA-5-1	51.14%	38.55%	42.53%	35.75%
884	Val-CAC-2-1	44.11%	37.73%	51.67%	53.84%
885	Thr-TGT-1-1	46.75%	36.77%	49.56%	56.01%
886	Arg-TCG-1-1	46.40%	36.14%	39.72%	52.06%
887	Lys-TTT-6-1	45.69%	35.77%	52.02%	57.66%
888	Arg-TCG-3-1	37.61%	27.54%	52.20%	61.17%

FIGURE 32

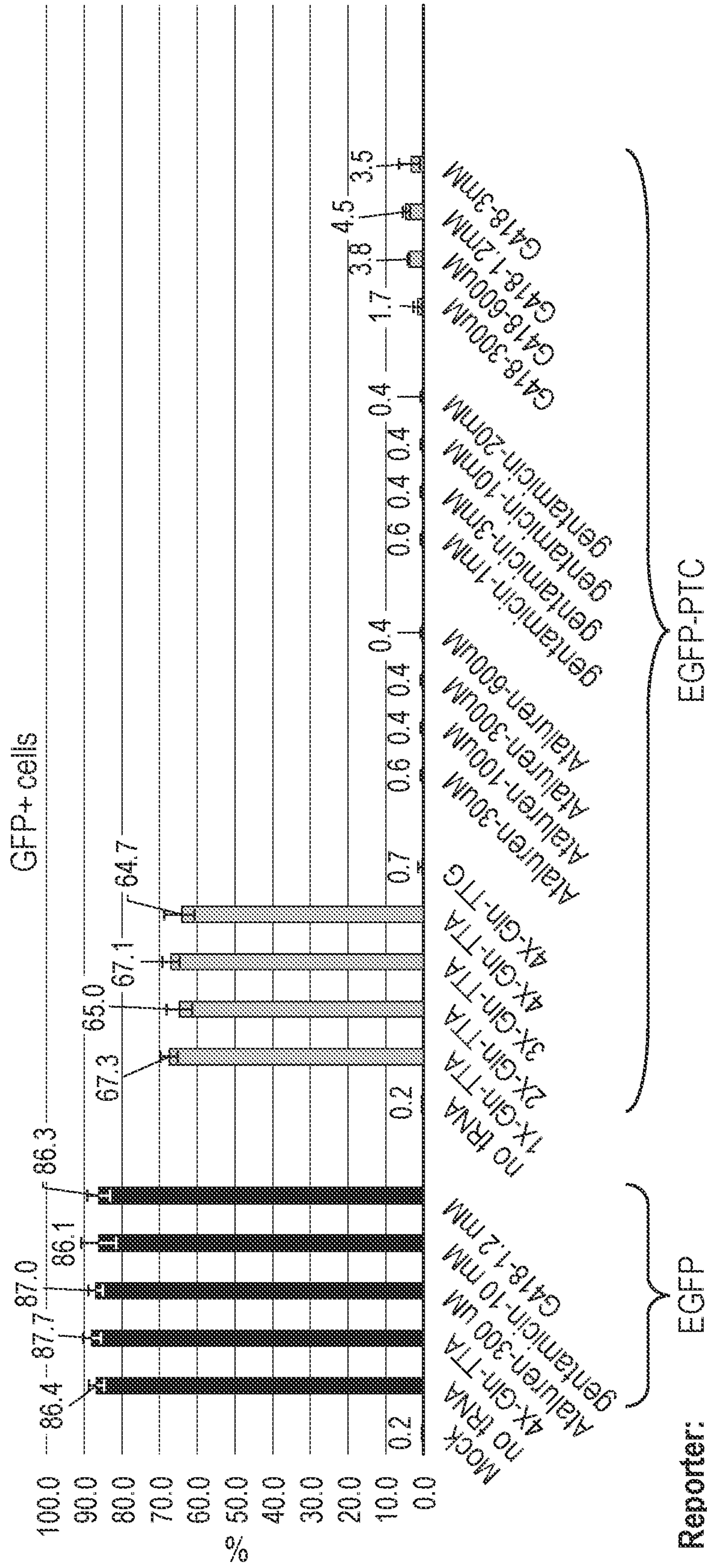


FIGURE 33

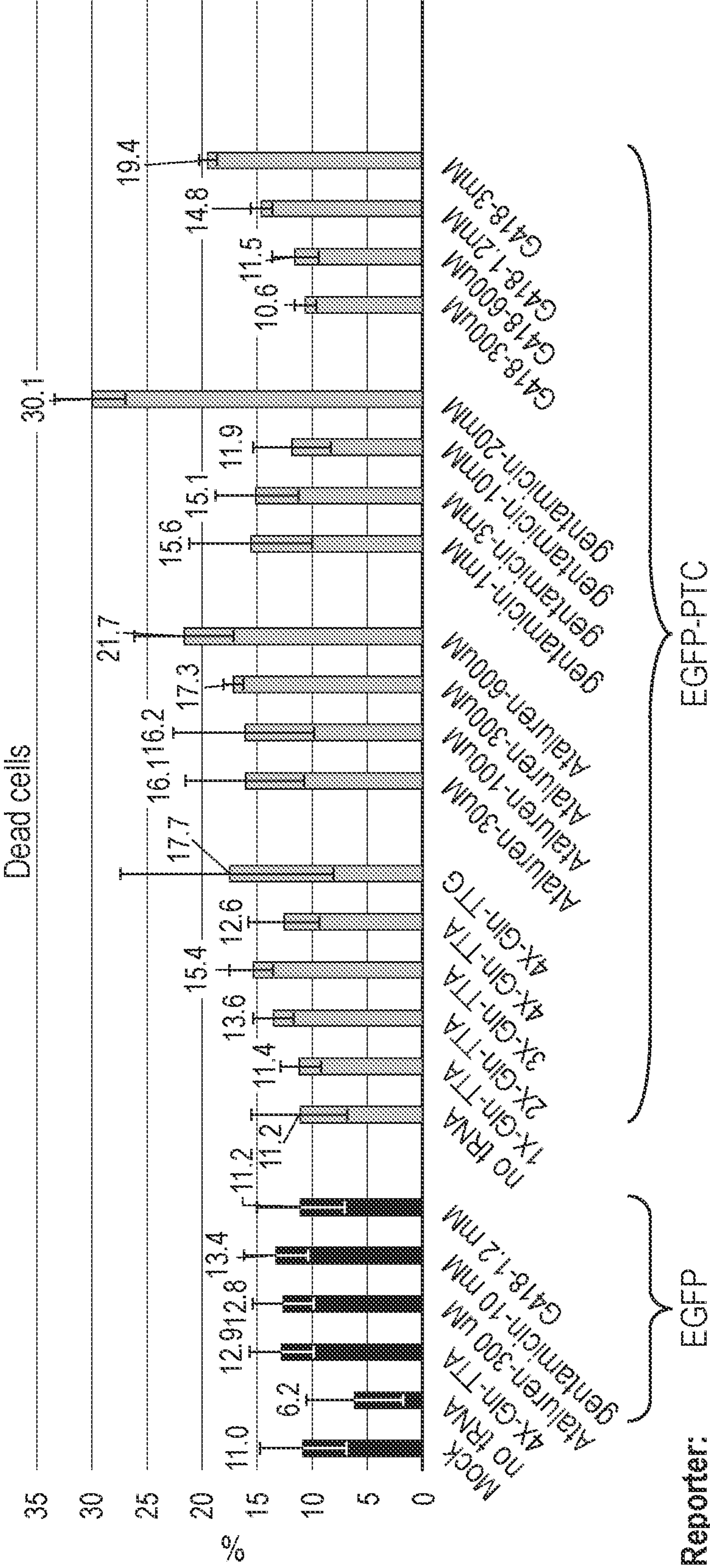
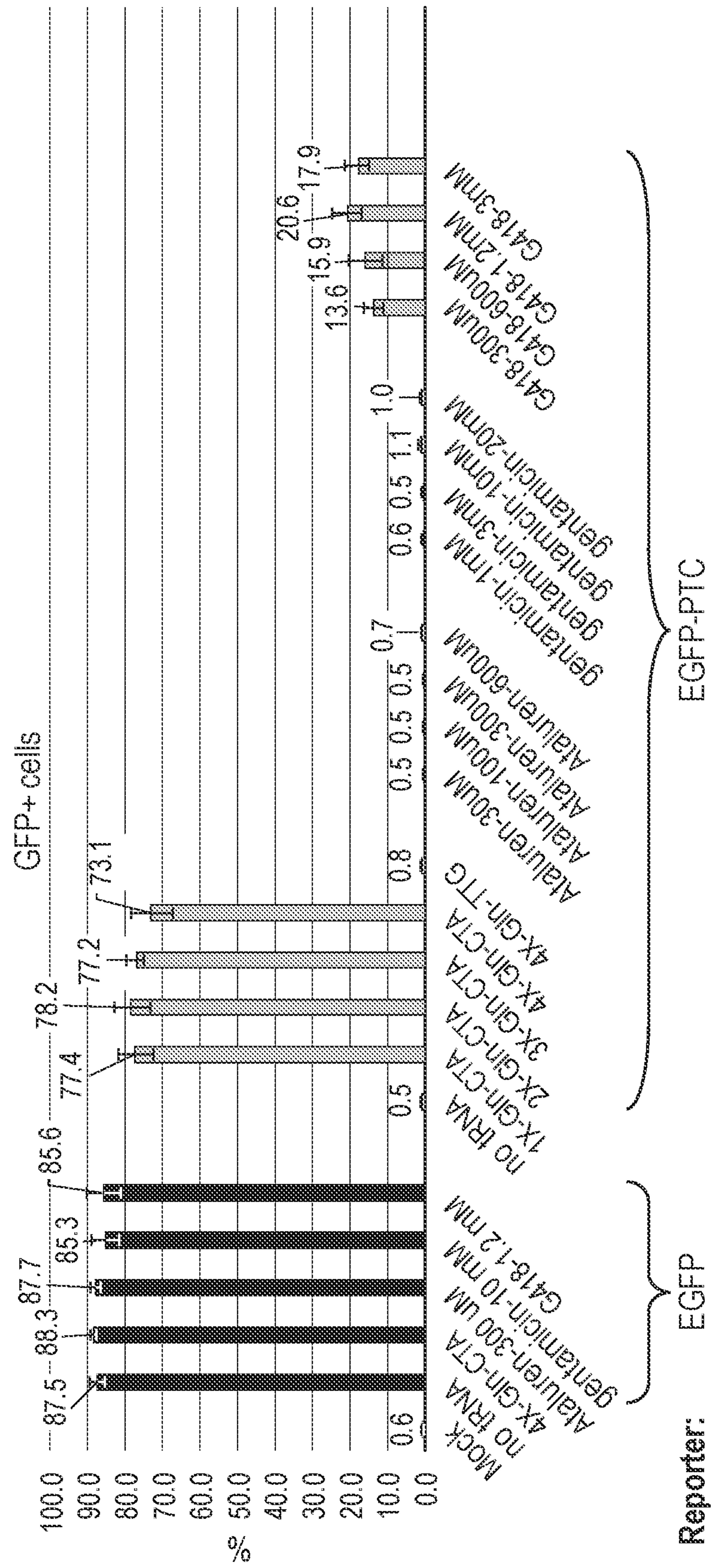


FIGURE 34



5346

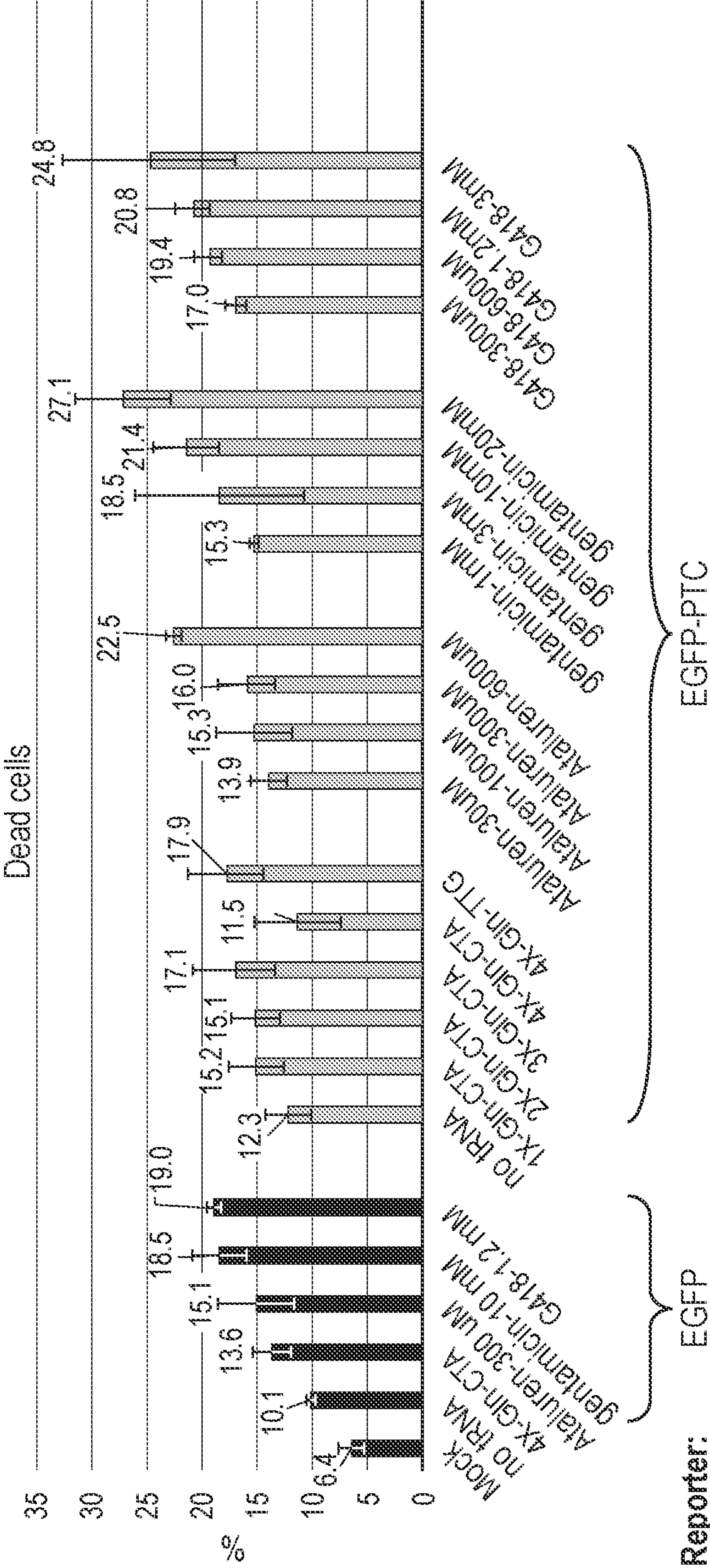


FIGURE 36

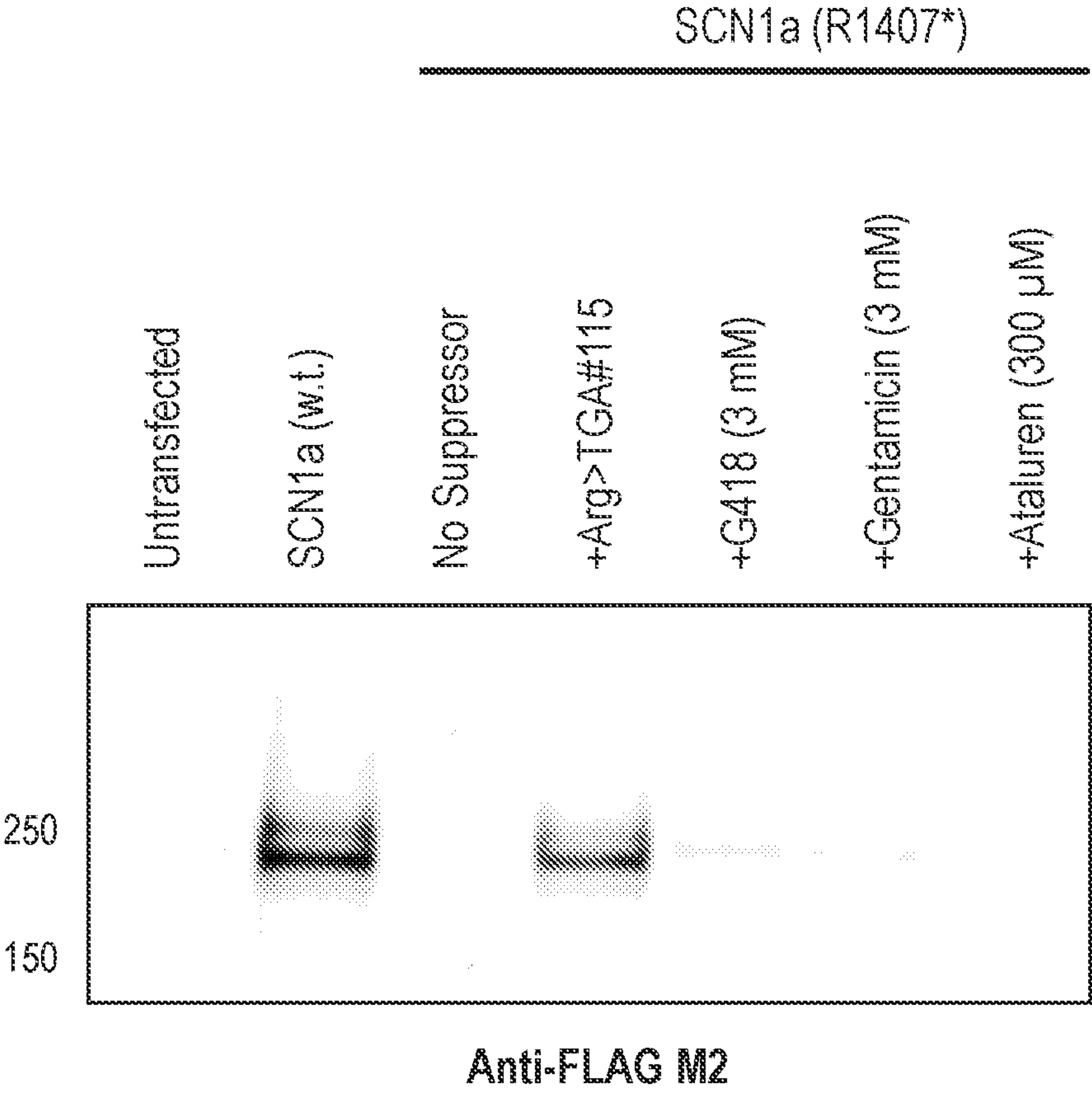


FIGURE 37A

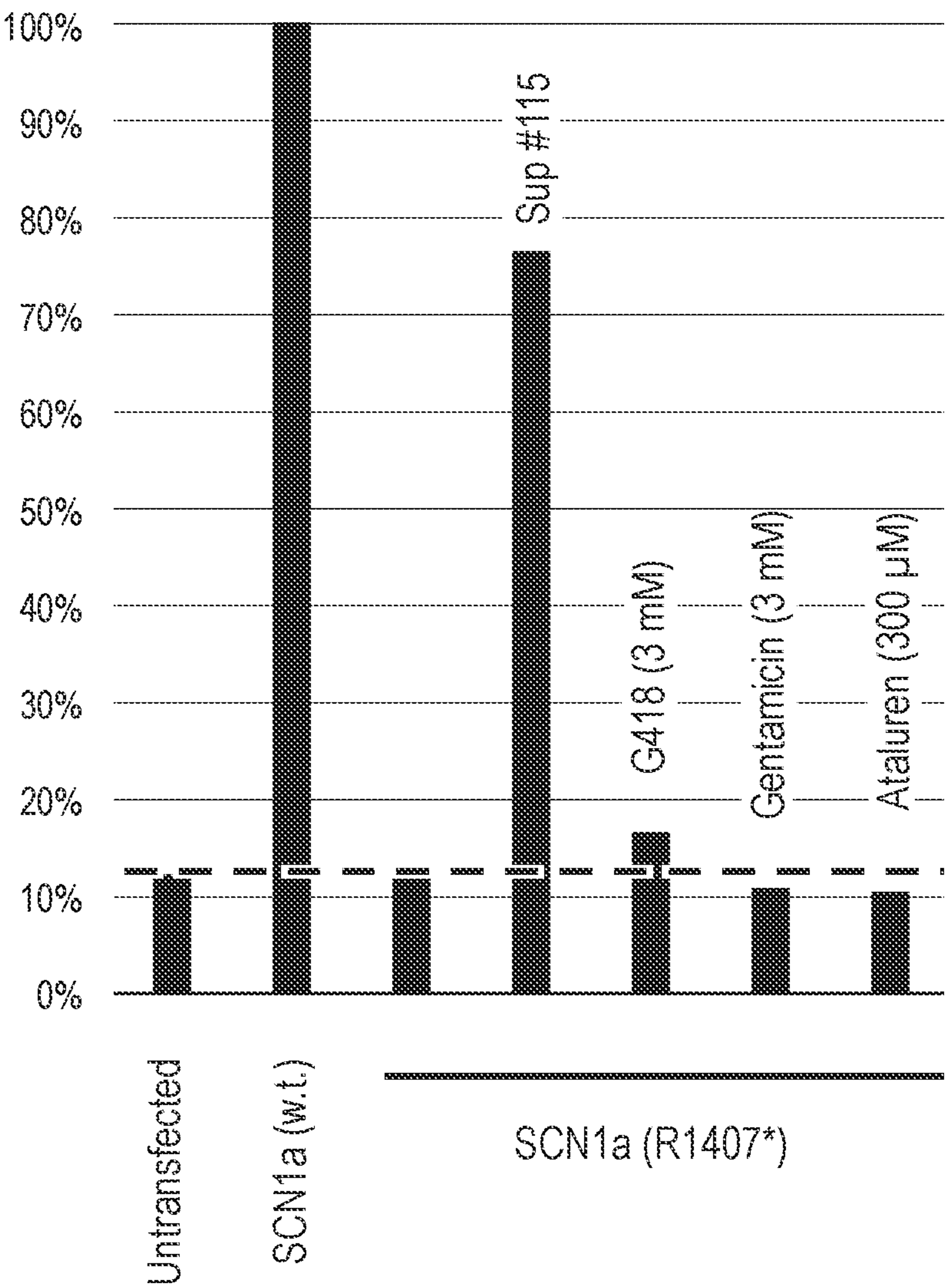


FIGURE 37B

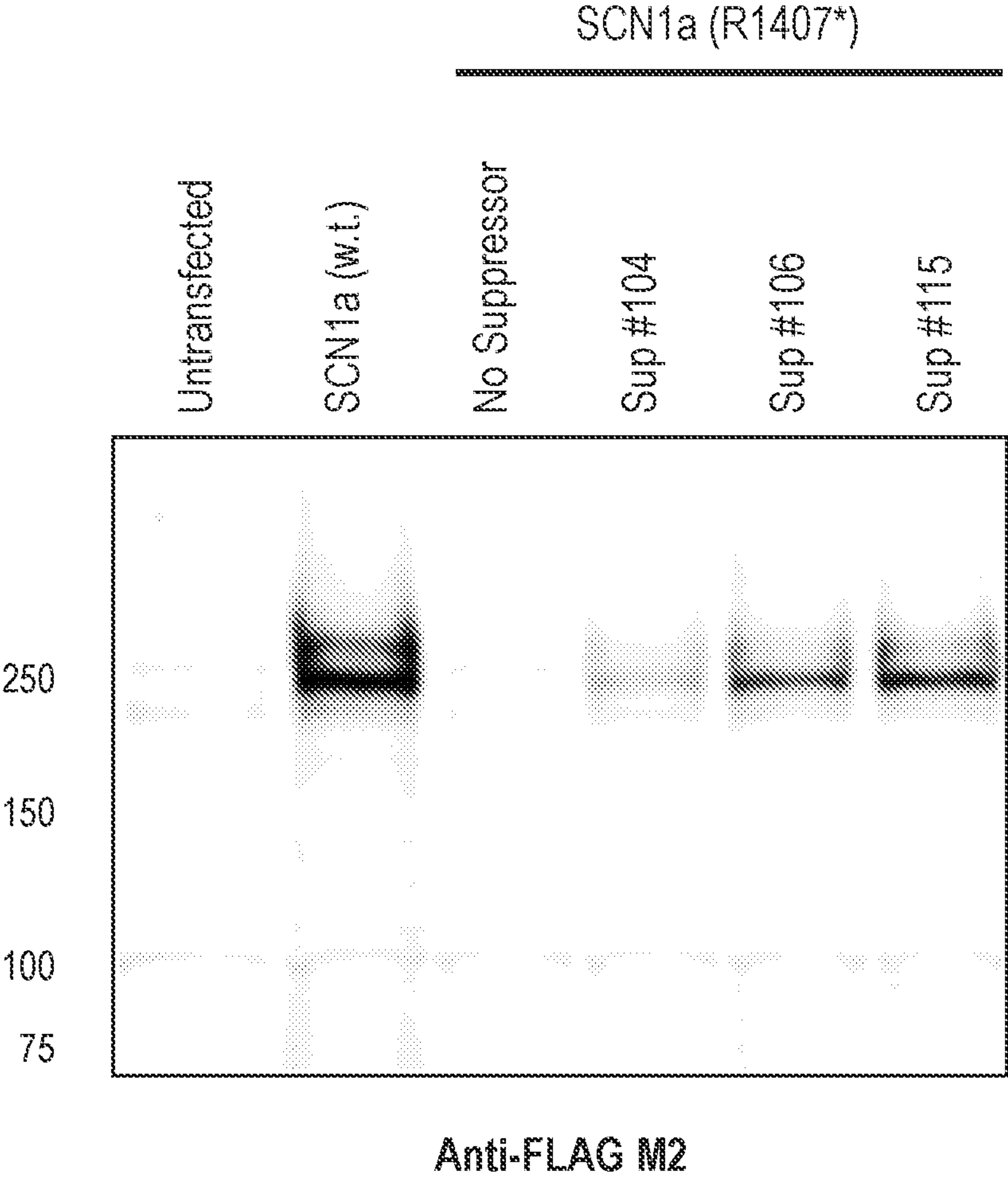


FIGURE 38A

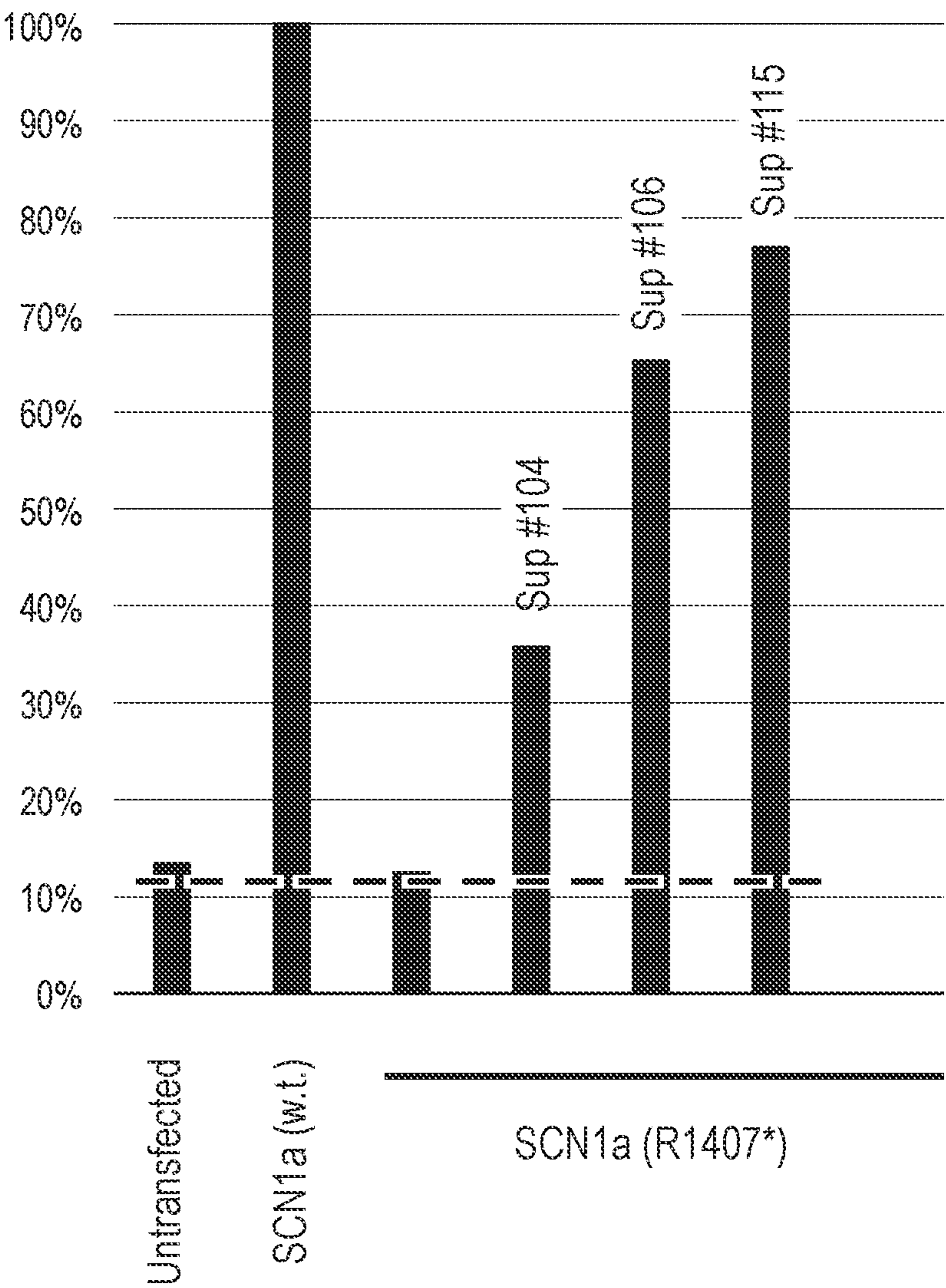


FIGURE 38B

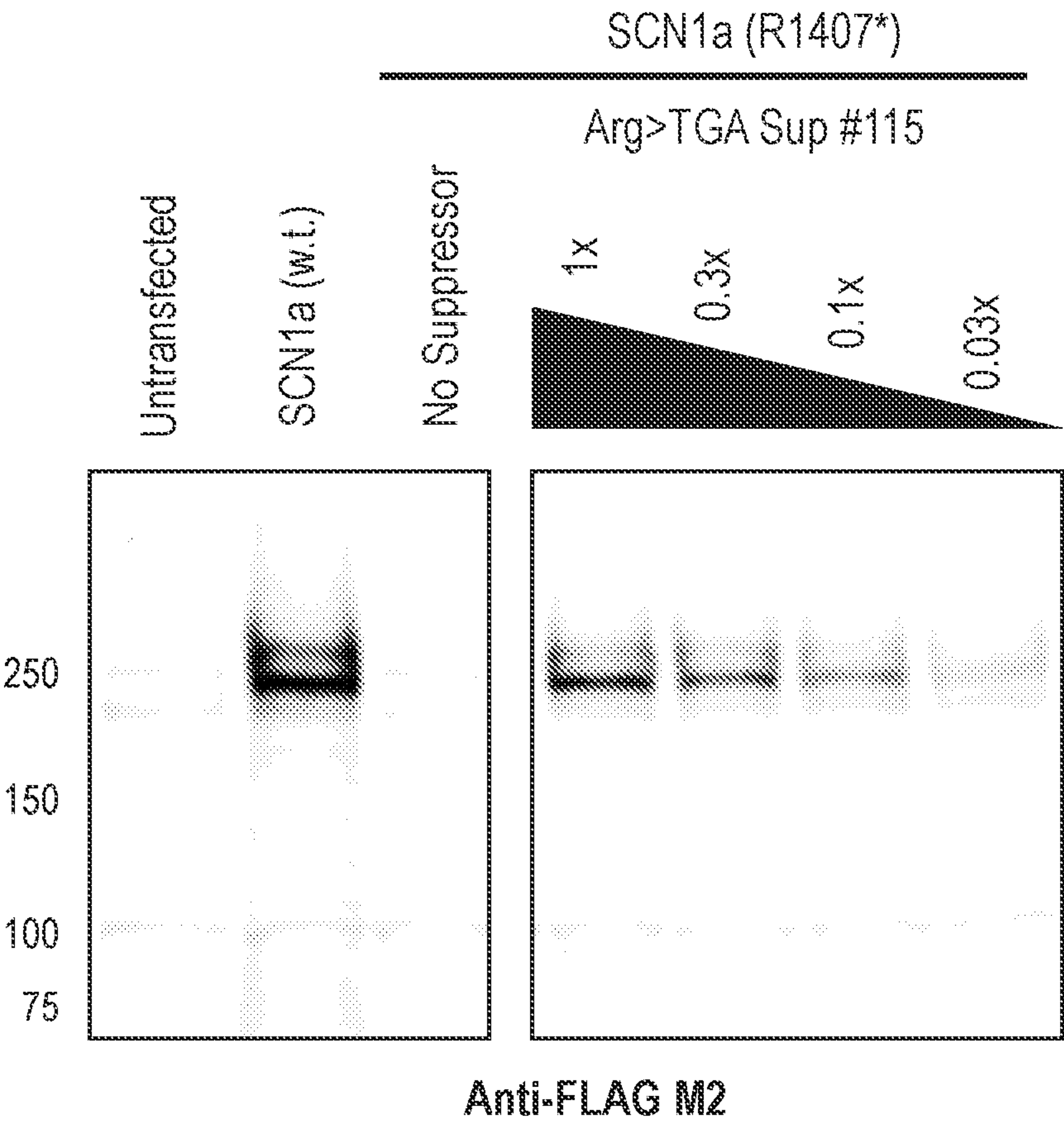


FIGURE 39A

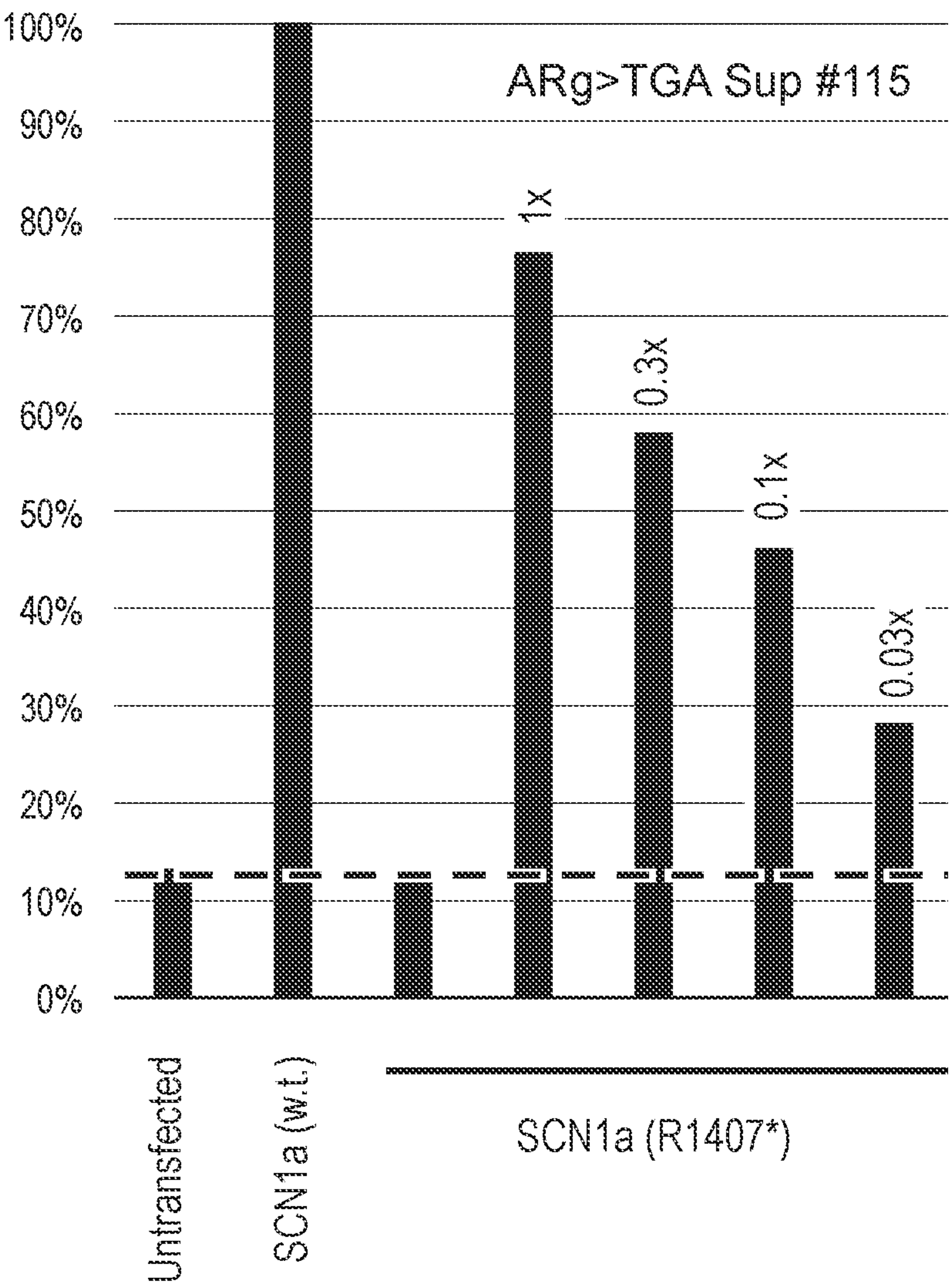


FIGURE 39B

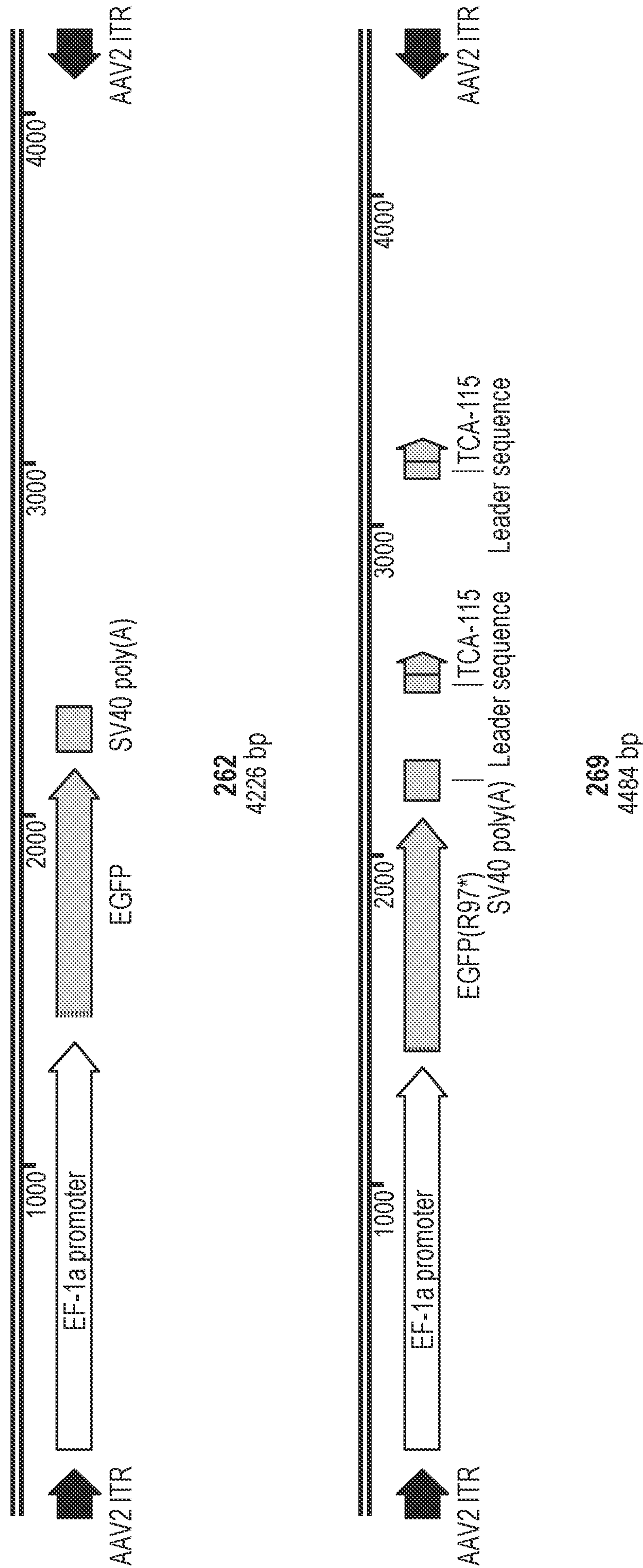


FIGURE 40

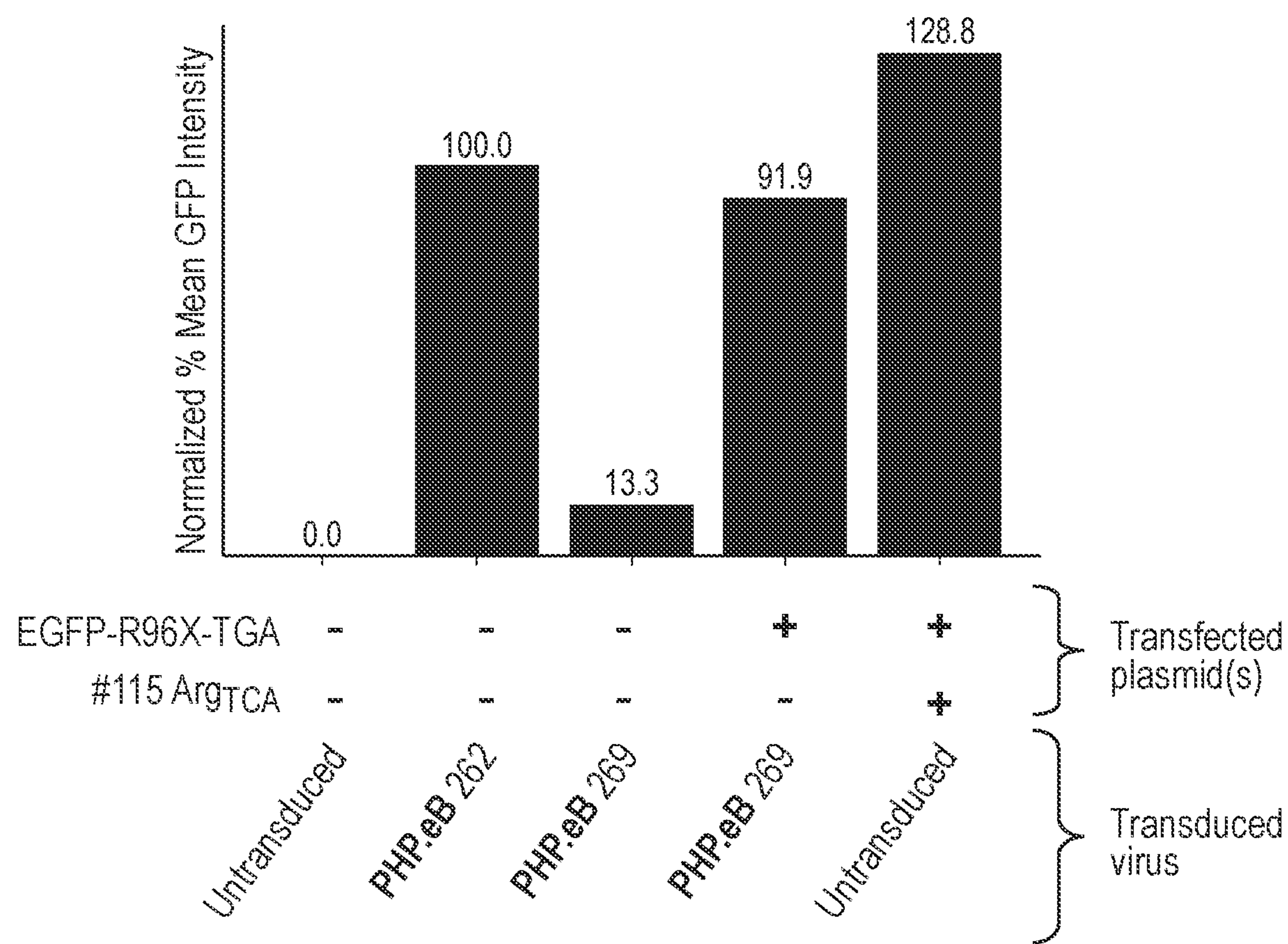


FIGURE 41

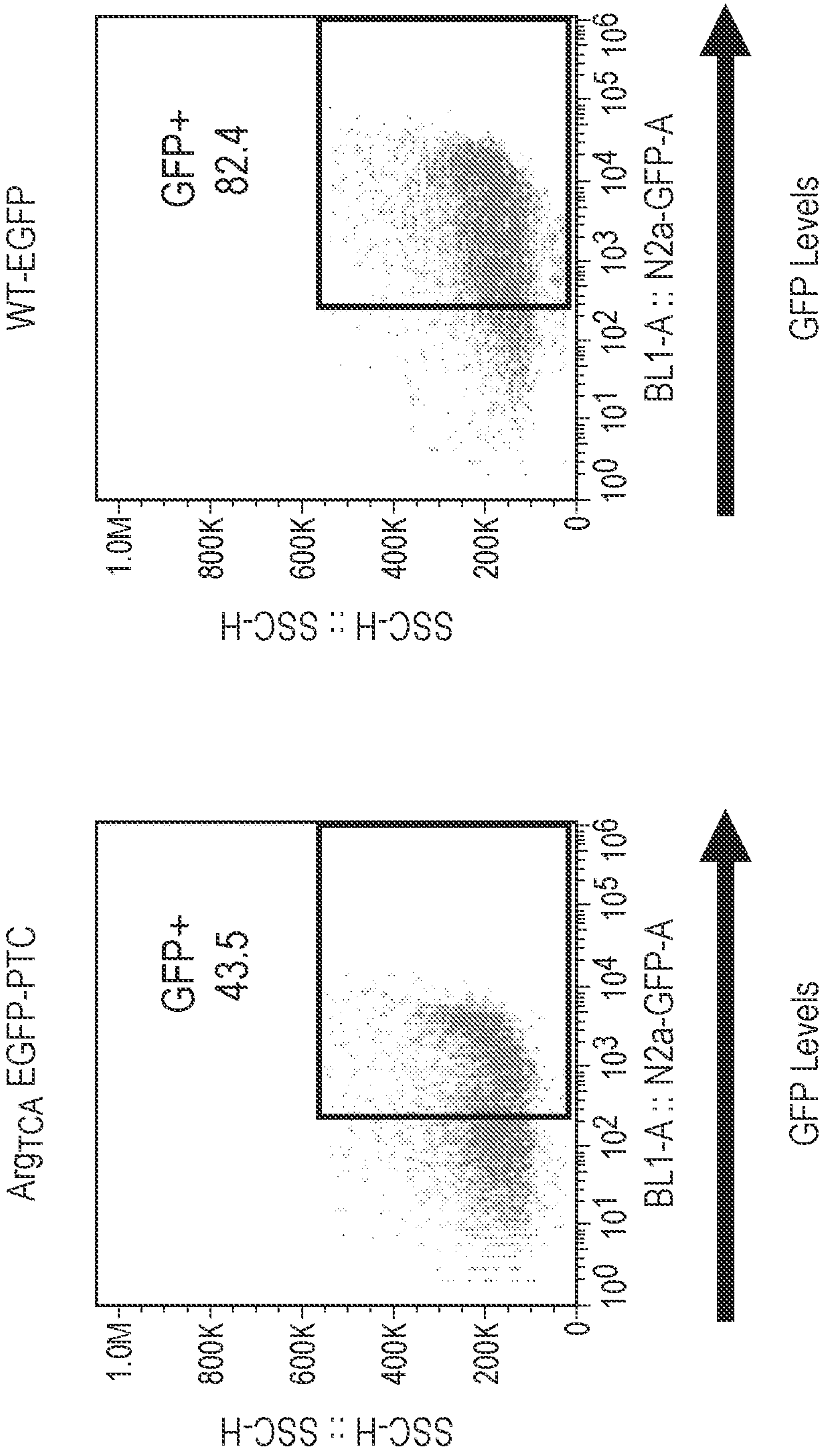


FIGURE 42

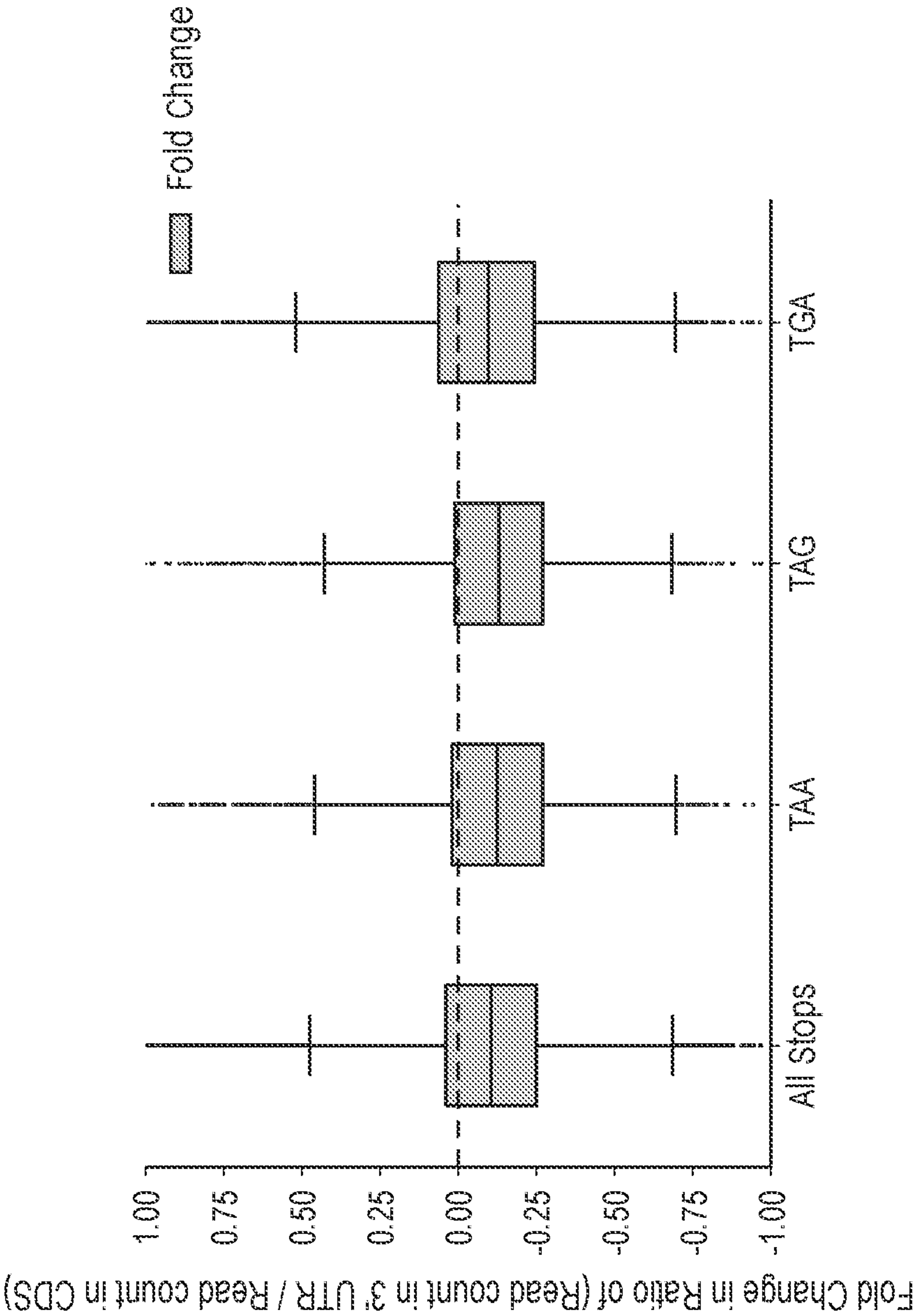


FIGURE 43

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/58415

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 31/7105, A61K 48/00, C12N 15/11 (2021.01)

CPC - C12N 15/113, A61K 31/7105, A61K 48/00, C12N 15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2019/090154 A1 (UNIVERSITY OF IOWA RESEARCH FOUNDATION) 09 May 2019 (09.05.2019) Claim 1, Claim 2, SEQ ID NO: 90	1, 3/1
A	US 2018/0320175 A1 (THE GENERAL HOSPITAL) 08 November 2018 (08.11.2018) abstract, para [0015], SEQ ID NO: 27719	1, 3/1

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

21 March 2021

Date of mailing of the international search report

APR 09 2021

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Lee Young

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/58415

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-8, 12-26, 29-30, 33-42, 45-50
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- see extra sheet for Box No. III Observations where unity of invention is lacking -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1 and 3(in part) limited to SEQ ID NO: 2

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/58415

Continuation of:

Box No. III. Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Groups I+: Claims 1-3, 9-11, drawn to an expression vector comprising a nucleotide sequence encoding a tRNA. The composition will be searched to the extent that the tRNA encompasses SEQ ID NO: 2 (the first nucleotide sequence listed in Table 2). It is believed that claims 1 and 3(in part) encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 2. Additional tRNA(s) and vector(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected tRNA(s) and vector(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a composition comprising tRNA SEQ ID NO: 1 and vector SEQ ID NO: 26 (Claims 9, 10)

Groups II+: Claims 27-28, 31-32, 43-44, drawn to a method of expressing in a mammalian cell a functional gene product encoded by a gene containing a premature termination codon, the method comprising introducing into the cell an effective amount of a tRNA, 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. Group II+ will be searched upon payment of additional fees. The method may be searched, for example, to the extent that tRNA encompasses SEQ ID NO: 1 for an additional fee and election as such. It is believed that claims 27, 31, 43 read on this exemplary invention. Additional tRNA(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected tRNA(s). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be a tRNA comprising SEQ ID NO: 6 (Claims 27-28, 31-32, 43-44).

The inventions listed as Groups I+ and II+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I+ include the special technical feature of a composition which differs from the special technical feature of a method, as disclosed by Groups II+.

No technical features are shared between the nucleotide sequences of Groups I+ and II+, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ and II+ were considered to share the technical features of including: an expression vector comprising a nucleotide sequence encoding a tRNA, these shared technical features are previously disclosed by WO 2019/090169 A1 to the Wistar Institute of Anatomy and Biology et al., (hereinafter "Wistar").

Wistar teaches (instant claim 9) an expression vector comprising a nucleotide sequence encoding a tRNA set forth in Table 3, wherein the expression vector comprises 1, 2, 3, 4, or more than 4 copy numbers of the nucleotide sequence encoding the tRNA (pg 7, ln 12-16, Figure 9. CFTR 1282x rescue with Trpchr17.trna39 and Glychr19.trna2 ACE-tRNAs. Biochemical western blot data of CFTR W1282X channels co-expressed in HEK cells with the indicated tRNA. Expression vectors containing four copies of the indicated tRNA display higher rescue of the CFTR protein.).

Wistar teaches (instant claim 27) a method of expressing in a mammalian cell a functional gene product encoded by a gene containing a premature termination codon, the method comprising introducing into the cell an effective amount of a tRNA, 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 (pg 5, ln 3-7, The present invention provides a method of treating a stop-codon-associated genetic disease, comprising administering the modified tRNA composition described above to a patient in need thereof. In certain embodiments, the genetic disease associated with a premature stop codon is cystic fibrosis, muscular dystrophy, P-thalassemia or Liddle's syndrome.; pg 13, ln 12-16, The current studies show that the specific site within an RNA transcript to which a tRNA delivers its amino acid can be changed through molecular editing of the anti-codon sequence within the tRNA. This approach allowed for a premature termination codon (PTC) to be effectively and therapeutically reverted back into the originally lost amino acid Anti-codon edited tRNA (ACE-tRNA) form a new class of biological therapeutics.).

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I+ and II+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.