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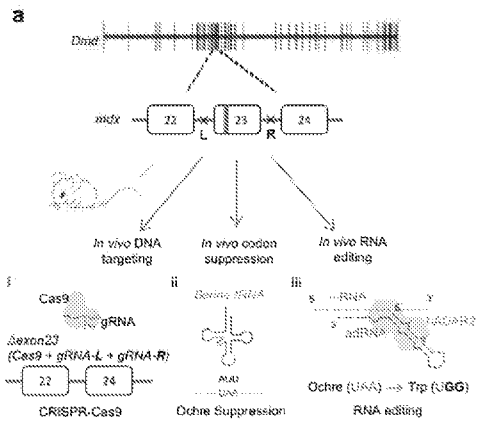
(54) Title: RNA TARGETING OF MUTATIONS VIA SUPPRESSOR tRNAs AND DEAMINASES

FIGURE 17



(57) Abstract: Aspects of the disclosure relate to a gene therapy approach for diseases, disorders, or conditions caused by mutation in the stop codon utilizing modified tRNA. At least 10-15% of all genetic diseases, including muscular dystrophy (e.g. Duchene muscular dystrophy), some cancers, beta thalassemia, Hurler syndrome, and cystic fibrosis, fall into this category. Not to be bound by theory, it is believed that this approach is safer than CRISPR approaches due to minimal off-target effects and the lack of genome level changes.

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RNA TARGETING OF MUTATIONS VIA SUPPRESSOR tRNAs AND DEAMINASES

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority under 35 U.S.C. 119(e) to U.S. Serial No. 62/466,961, filed March 3, 2017, and U.S. Serial No. 62/551,732, filed August 29, 2017, the entirety of which are incorporated by reference herein.

STATEMENT REGARDING GOVERNMENT SUPPORT

[0002] This invention was made with government support under Grant No. R01HG009285 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0003] Aspects of the disclosure relate to a gene therapy approach for diseases, disorders, or conditions caused by mutation in the stop codon using modified tRNA. At least 10-15% of all genetic diseases, including muscular dystrophy *(e.g.* Duchene muscular dystrophy), some cancers, beta thalassemia, Hurler syndrome, and cystic fibrosis, fall into this category. Not to be bound by theory, it is believed that this approach is safer than CRISPR or TALEN approaches due to minimal off-target effects and the lack of genome level changes.

SUMMARY

[0004] Aspects of the disclosure relate to a method for restoring expression of a protein comprising a point mutation in an RNA sequence encoding the protein in a subject in need thereof, the method comprising, or alternatively consisting essentially of, or yet further consisting of administering to the subject a vector encoding one or more tRNA having an anticodon sequence that recognizes a codon comprising the point mutation to the subject, optionally wherein the point mutation results in a premature stop codon, optionally wherein the point mutation results in a premature stop codon. In some embodiments, the point mutation results in a nonsense mutation having the DNA sequence TAA and the RNA

sequence UAA. In some embodiments, the tRNA is an endogenous tRNA with a modified anticodon stem recognizing the codon comprising the point mutation. In further embodiments, the tRNA is charged with a serine. In some embodiments, the tRNA is an orthogonal tRNA charged with a non-canonical amino acid. In further embodiments, the vector further comprises a corresponding tRNA synthetase. In some embodiments, the corresponding synthetase is E. coli Glutaminyl-tRNA synthetase. In some embodiments involving an orthogonal tRNA, the non-canonical amino acid is pyrrolysine. In further embodiments, the pyrrolysine is administered to the subject by introduction into the diet of the subject. In some embodiments, the vector encodes two tRNA having an anticodon sequence that recognizes the codon comprising the point mutation. In some embodiments, the protein is dystrophin. In a further aspect, the subject is a human and is optionally a pediatric patient.

[0005] Further method aspects relate to a treating a disease, disorder, or condition characterized by the presence of a point mutation in an RNA sequence encoding a protein associated with the disease, disorder, or condition in a subject in need thereof, the method comprising, or alternatively consisting essentially of, or yet further consisting of, administering to the subject a vector encoding one or more tRNA having an anticodon sequence that recognizes a codon comprising the point mutation to the subject, optionally wherein the point mutation results in a premature stop codon. In some embodiments, the point mutation results in a nonsense mutation having the DNA sequence TAA and the RNA sequence UAA. In some embodiments, the tRNA is an endogenous tRNA with a modified anticodon stem recognizing the codon comprising the point mutation. In further embodiments, the tRNA is charged with a serine. In some embodiments, the tRNA is an orthogonal tRNA charged with a non-canonical amino acid. In further embodiments, the vector further comprises a corresponding tRNA synthetase. In some embodiments, the corresponding synthetase is E. coli Glutaminyl-tRNA synthetase. In some embodiments involving an orthogonal tRNA, the non-canonical amino acid is pyrrolysine. In further embodiments, the pyrrolysine is introduced in the diet of the subject. In some embodiments, the vector encodes two tRNA having an anticodon sequence that recognizes the codon comprising the point mutation. In some embodiments, the disease, disorder, or condition is

selected from the group consisting of the diseases, disorders, and conditions listed in **Table 1**, optionally characterized by the presence of a nonsense mutation and/or a premature stop codon. In some embodiments, the protein is dystrophin. In further embodiments, the disease, disorder, or condition is muscular dystrophy. In still further embodiments, the disease disorder or condition is Duchenne muscular dystrophy. In some embodiments, the subject is a human and is optionally a pediatric patient.

[0006] Still further aspects disclosed herein relate to a vector encoding one or more tRNA having an anticodon sequence that recognizes a codon comprising a point mutation in an RNA sequence encoding a protein, optionally wherein the point mutation results in a premature stop codon. In some embodiments, the point mutation results in a nonsense mutation having the DNA sequence TAA and the RNA sequence UAA. In some embodiments, the tRNA is an endogenous tRNA with a modified anticodon stem recognizing the codon comprising the point mutation. In further embodiments, the tRNA is charged with a serine. In some embodiments, the tRNA is an orthogonal tRNA charged with a non-canonical amino acid. In further embodiments, the vector further comprises a corresponding tRNA synthetase. In some embodiments, the corresponding synthetase is E. coli Glutaminyl-tRNA synthetase. In some embodiments involving an orthogonal tRNA, the non-canonical amino acid is pyrrolysine. In some embodiments, the vector encodes two tRNA having an anticodon sequence that recognizes the codon comprising the point mutation. In some embodiments, the vector is an AAV vector, optionally an AAV8 vector. In some embodiments, the protein is dystrophin. In a further aspect, the subject is a human and is optionally a pediatric patient.

[0007] In another aspect, the disclosure relates to a method for restoring expression of a protein comprising a point mutation in an RNA sequence encoding the protein in a subject in need thereof comprising administering one or more vectors encoding an ADAR based RNA editing system comprising one or more forward guide RNAs for the ADAR ("adRNAs") and one or more corresponding reverse guide RNAs for the ADAR ("radRNAs") to the subject, wherein the ADAR based RNA editing system specifically edits the point mutation. In some embodiments, the point mutation results in a nonsense mutation having the DNA sequence TAA and the RNA sequence UAA. In some embodiments, the ADAR based RNA editing

system converts UAA to UIA and, optionally, further UIA to UII. In some embodiments, the ADAR based RNA editing system converts UAA to UAL In some embodiments, optionally those involving nonsense or missense mutations, the RNA targeted in mRNA. In further embodiments, the one or more vector further encodes a tRNA that targets an amber codon. In some embodiments, the protein is dystrophin. In some embodiments, the point mutation results in a splice site or missense mutation having the DNA sequence CAG and the RNA sequence CAG. In some embodiments, the ADAR based RNA editing system converts CAG to CIG. In some embodiments, optionally those involving splice site mutations, the RNA targeted is pre-mRNA. In some embodiments, the protein is ornithine transcabamylase. In some embodiments, the ADAR based editing system further comprises ADARl, ADAR2, the E488Q and E100Q mutants each thereof, a fusion protein comprising the catalytic domain of an ADAR and a domain which associates with an RNA hairpin motif, a fusion protein comprising the catalytic domain of an ADAR and a dead Cas9, or a fusion protein comprising the double stranded binding domain of an ADAR and an APOBEC. In further embodiments, the domain which associates with an RNA hairpin motif is selected from the group of an MS2 bacteriophage coat protein (MCP) and an N22 peptide. In some embodiments, the method further comprises administering an effective amount of an interferon to enhance endogenous ADARl expression. In still further embodiments, the interferon is interferon a. In some embodiments, the adRNA comprises one or more RNA hairpin motifs. In some embodiments, the one or more RNA hairpin motifs are selected from the group of an MS2 stem loop and a BoxB loop and/or are stabilized by replacing A-U with G-C. In some embodiments, the adRNA is stabilized through the incorporation of one or more of 2'-0-methyl, 2'-0-methyl 3'phosphorothioate, or 2'-0-methyl 3'thioPACE at either or both termini of the adRNA. In a further aspect, the subject is a human and is optionally a pediatric patient.

[0008] Further method aspects relate to a method of treating a disease, disorder, or condition characterized by the presence of a point mutation in an RNA sequence encoding a protein associated with the disease, disorder, or condition in a subject in need thereof, the method comprising, or alternatively consisting essentially of, or yet further consisting of, administering to the subject one or more vectors encoding an ADAR based RNA editing system comprising one or more forward guide RNAs for the ADAR ("adRNAs") and one or

more corresponding reverse guide RNAs for the ADAR ("radRNAs") to the subject, wherein the ADAR based RNA editing system specifically edits the point mutation. In some embodiments, the point mutation results in a nonsense mutation having the DNA sequence TAA and the RNA sequence UAA. In some embodiments, the ADAR based RNA editing system converts UAA to UIA and, optionally, further UIA to UII. In some embodiments, the ADAR based RNA editing system converts UAA to UAL In some embodiments, optionally those involving nonsense or missense mutations, the RNA targeted in mRNA. In further embodiments, the one or more vector further encodes a tRNA that targets an amber codon. In some embodiments, the protein is dystrophin. In some embodiments, the point mutation results in a splice site or missense mutation having the DNA sequence CAG and the RNA sequence CAG. In some embodiments, the ADAR based RNA editing system converts CAG to CIG. In some embodiments, optionally those involving splice site mutations, the RNA targeted is pre-mRNA. In some embodiments, the protein is ornithine transcabamylase. In some embodiments, the ADAR based editing system further comprises ADARl, ADAR2, the E488Q and E100Q mutants each thereof, a fusion protein comprising the catalytic domain of an ADAR and a domain which associates with an RNA hairpin motif, a fusion protein comprising the catalytic domain of an ADAR and a dead Cas9, or a fusion protein comprising the double stranded binding domain of an ADAR and an APOBEC. In further embodiments, the domain which associates with an RNA hairpin motif is selected from the group of an MS2 bacteriophage coat protein (MCP) and an N22 peptide. In some embodiments, the method further comprises administering an effective amount of an interferon to enhance endogenous ADARl expression. In still further embodiments, the interferon is interferon a. In some embodiments, the adRNA comprises one or more RNA hairpin motifs. In some embodiments, the one or more RNA hairpin motifs are selected from the group of an MS2 stem loop and a BoxB loop and/or are stabilized by replacing A-U with G-C. In some embodiments, the adRNA is stabilized through the incorporation of one or more of 2'-0-methyl, 2'-0-methyl 3'phosphorothioate, or 2'-0-methyl 3'thioPACE at either or both termini of the adRNA. In some embodiments, the disease, disorder, or condition is selected from the group consisting of the diseases, disorders, and conditions listed in **Table 1**. In further embodiments, the protein is dystrophin and the disease, disorder, or condition is muscular dystrophy. In still further embodiments, the disease disorder or condition is Duchenne

muscular dystrophy. In some embodiments, the subject is a human and is optionally a pediatric patient.

[0009] Additional aspects relate to a recombinant expression system comprising one or more vectors encoding an ADAR based RNA editing system comprising one or more forward guide RNAs for the ADAR ("adRNAs") and one or more corresponding reverse guide RNAs for the ADAR ("radRNAs") to the subject, wherein the ADAR based RNA editing system specifically edits a point mutation in an RNA sequence encoding a protein. In some embodiments, the point mutation results in a nonsense mutation having the DNA sequence TAA and the RNA sequence UAA. In some embodiments, the ADAR based RNA editing system converts UAA to UIA and, optionally, further UIA to UII. In some embodiments, the ADAR based RNA editing system converts UAA to UAL In some embodiments, optionally those involving nonsense or missense mutations, the RNA targeted in mRNA. In further embodiments, the one or more vector further encodes a tRNA that targets an amber codon. In some embodiments, the protein is dystrophin. In some embodiments, the point mutation results in a splice site or missense mutation having the DNA sequence CAG and the RNA sequence CAG. In some embodiments, the ADAR based RNA editing system converts CAG to CIG. In some embodiments, optionally those involving splice site mutations, the RNA targeted is pre-mRNA. In some embodiments, the protein is ornithine transcabamylase. In some embodiments, the ADAR based editing system further comprises ADARl, ADAR2, the E488Q and E100Q mutants each thereof, a fusion protein comprising the catalytic domain of an ADAR and a domain which associates with an RNA hairpin motif, a fusion protein comprising the catalytic domain of an ADAR and a dead Cas9, or a fusion protein comprising the double stranded binding domain of an ADAR and an APOBEC. In further embodiments, the domain which associates with an RNA hairpin motif is selected from the group of an MS2 bacteriophage coat protein (MCP) and an N22 peptide. In some embodiments, the adRNA comprises one or more RNA hairpin motifs. In some embodiments, the one or more RNA hairpin motifs are selected from the group of an MS2 stem loop and a BoxB loop and/or are stabilized by replacing A-U with G-C. In some embodiments, the adRNA is stabilized through the incorporation of one or more of 2'-**0**-methyl, 2'-**0**-methyl 3'phosphorothioate, or

2'-0-methyl 3'thioPACE at either or both termini of the adRNA. In a further aspect, the subject is a human and is optionally a pediatric patient.

[0010] Still further aspects relate to a composition comprising any one or more of the vectors disclosed herein and optionally one or more carriers, such as a pharmaceutically acceptable carrier. In some embodiments, the composition further comprises an effective amount of an interferon to enhance endogenous ADAR1 expression. In still further embodiments, the interferon is interferon a.

[0011] Some aspects disclosed herein relate to methods for restoring expression of a protein in a subject in need thereof, the method comprising, or alternatively consisting essentially of, or yet further consisting of, administering to the subject a tRNA having an anticodon sequence that recognizes a mutation in an RNA sequence encoding the protein or a vector encoding one or more of said tRNA to the subject. In some embodiments, the mutation is a nonsense mutation, optionally a premature stop codon. In some embodiments, the nonsense mutation is TAA in DNA and UAA in RNA. In some embodiments, the tRNA is a modified endogenous tRNA charged with a canonical amino acid. In some embodiments, the canonical amino acid is serine. In some embodiments, the tRNA is an orthogonal tRNA charged with a non-canonical amino acid. In some embodiments, the orthogonal tRNA has a corresponding synthetase. In some embodiments, the corresponding synthetase is E. coli Glutaminyl-tRNA synthetase. In some embodiments, the non-canonical amino acid is introduced or administered to the subject *(e.g.* through food), allowing for the induction of the orthogonal tRNA activity. In some embodiments, the non-canonical amino acid is pyrrolysine. In some embodiments, the tRNA targets an amber codon. In some embodiments, the tRNA targets an ochre codon. In some embodiments, the tRNA targets an opal codon. In some embodiments, the protein is dystrophin. In a further aspect, the subject is a human and is optionally a pediatric patient.

[0012] Further aspects disclosed herein relate to methods of a disease, disorder, or condition characterized by a protein deficiency in a subject in need thereof, the method comprising, or alternatively consisting essentially or, or yet further consisting of administering a tRNA having an anticodon sequence that recognizes a mutation in an RNA

sequence encoding the protein or a vector encoding one or more of said tRNA to the subject. In some embodiments, the mutation is a nonsense mutation, optionally a premature stop codon. In some embodiments, the nonsense mutation is TAA in DNA and UAA in RNA. In some embodiments, the tRNA is a modified endogenous tRNA charged with a canonical amino acid. In some embodiments, the canonical amino acid is serine. In some embodiments, the tRNA is an orthogonal tRNA charged with a non-canonical amino acid. In some embodiments, the orthogonal tRNA has a corresponding synthetase. In some embodiments, the corresponding synthetase is E. coli Glutaminyl-tRNA synthetase. In some embodiments, the non-canonical amino acid is administered or introduced to the subject *(e.g.* through food), allowing for the induction of the orthogonal tRNA activity. In some embodiments, the non-canonical amino acid is pyrrolysine. In some embodiments, the tRNA targets an amber codon. In some embodiments, the tRNA targets an ochre codon. In some embodiments, the tRNA targets an opal codon. In some embodiments, the protein deficiency is a dystrophin deficiency. In some embodiments, the disease, disorder, or condition is muscular dystrophy. In some embodiments, the muscular dystrophy is Duchene muscular dystrophy. In a further aspect, the subject is a human and is optionally a pediatric patient.

[0013] Other aspects relate to a vector encoding one or more tRNA having an anticodon sequence that recognizes a mutation in an RNA sequence encoding the protein. In some embodiments, the mutation is a nonsense mutation, optionally a premature stop codon. In some embodiments, the nonsense mutation is TAA in DNA and UAA in RNA. In some embodiments, the tRNA is a modified endogenous tRNA charged with a canonical amino acid. In some embodiments, the canonical amino acid is serine. In some embodiments, the tRNA is an orthogonal tRNA charged with a non-canonical amino acid. In some embodiments, the orthogonal tRNA has a corresponding synthetase. In some embodiments, the corresponding synthetase is E. coli Glutaminyl-tRNA synthetase. In some embodiments, the vector further comprises the corresponding synthetase. In some embodiments, the non-canonical amino acid is introduced or administered to the subject *(e.g.* through food), allowing for the induction of the orthogonal tRNA activity. In some embodiments, the non-canonical amino acid is pyrrolysine. In some embodiments, the tRNA targets an amber codon. In some embodiments, the tRNA targets an ochre codon. In some embodiments, the

tRNA targets an opal codon. In some embodiments, the protein is dystrophin. In some embodiments, the mutation is a nonsense mutation, optionally a premature stop codon. In some embodiments, the vector is an Adeno-Associated Virus (AAV) vector. In some embodiments, the AAV vector is an AAV8 vector.

[0014] Additional aspects of this disclosure relate to on-demand, in vivo production of therapeutic proteins, such as, but not limited to, (i) insulin; (ii) neutralizing antibodies for viruses *(e.g.* HIV, HCV, HPV, influenza) and bacteria *(e.g. Staph Aureus;* drug resistant strains). Such method aspects comprise administering to a subject a vector encoding the therapeutic protein with a mutation in its sequence and a tRNA having an anticodon sequence that recognizes the mutation in the RNA sequence encoding the therapeutic protein or a vector encoding one or more of said tRNA. Accordingly, any of the methods and vectors disclosed hereinabove relating to a tRNA having an anticodon sequence that recognizes a mutation in an RNA sequence encoding the protein or a vector encoding one or more of said tRNA may be applied to this aspect, as well.

[0015] Some aspects disclosed herein relate to methods for restoring expression of a protein in a subject in need thereof comprising administering an ADAR2 based RNA editing system comprising an ADAR2, one or more forward guide RNAs for the ADAR2 ("adRNAs"), and one or more corresponding reverse guide RNAs for the ADAR2 ("radRNAs") to the subject, wherein the ADAR2 based RNA editing system specifically edits a mutation in an RNA sequence encoding the protein or one or more vectors encoding said ADAR2, adRNAs, radRNAs. In some embodiments, the ADAR2 based RNA editing system changes adenosine (A) to inosine (I), which is read during translation as guanosine (G). In some embodiments, the mutation is a nonsense mutation. In some embodiments, the nonsense mutation is TAA in DNA and UAA in RNA. In some embodiments, the ADAR2 based RNA editing system causes point mutations at one or more adenosines (A) in the nonsense mutation. In some embodiments, the ADAR2 based RNA editing system converts UAA to UIA (read as UGA). In further embodiments, the ADAR2 based RNA editing system converts UIA (read as UGA) to UII (read as UGG). In some embodiments, the ADAR2 based RNA editing system converts UAA to UAI (read as UAG). In some embodiments, the method further comprises administering a tRNA, such as one disclosed hereinabove, that recognizes the codon encoded

by the ADAR2 edited sequence. In some embodiments, the tRNA is a modified endogenous tRNA charged with a canonical amino acid. In some embodiments, the canonical amino acid is serine. In some embodiments, the tRNA is an orthogonal tRNA charged with a non-canonical amino acid. In some embodiments, the orthogonal tRNA has a corresponding synthetase. In some embodiments, the corresponding synthetase is E. coli Glutaminyl-tRNA synthetase. In some embodiments, the non-canonical amino acid is introduced to the subject *(e.g.* through food), allowing for the induction of the orthogonal tRNA activity. In some embodiments, the non-canonical amino acid is pyrrolysine. In some embodiments, the tRNA targets an amber codon. In some embodiments, the tRNA targets an ochre codon. In some embodiments, the tRNA targets an opal codon. In some embodiments, the protein deficiency is a dystrophin deficiency. In some embodiments, the disease, disorder, or condition is muscular dystrophy. In some embodiments, the muscular dystrophy is Duchene muscular dystrophy.

[0016] Further aspects disclosed herein relate to methods of a disease, disorder, or condition characterized by a protein deficiency in a subject in need thereof comprising administering an ADAR2 based RNA editing system comprising an ADAR2, one or more forward guide RNAs for the ADAR2 ("adRNAs"), and one or more corresponding reverse guide RNAs for the ADAR2 ("radRNAs") to the subject, wherein the ADAR2 based RNA editing system specifically edits a mutation in an RNA sequence encoding the protein or one or more vectors encoding said ADAR2, adRNAs, radRNAs. In some embodiments, the ADAR2 based RNA editing system changes adenosine (A) to inosine (I), which is read during translation as guanosine (G). In some embodiments, the mutation is a nonsense mutation. In some embodiments, the nonsense mutation is TAA. In some embodiments, the ADAR2 based RNA editing system causes point mutations at one or more adenosines (A) in the nonsense mutation. In some embodiments, the ADAR2 based RNA editing system converts UAA to UIA (read as UGA). In further embodiments, the ADAR2 based RNA editing system converts UIA (read as UGA) to UII (read as UGG). In some embodiments, the ADAR2 based RNA editing system converts UAA to UAI (read as UAG). In some embodiments, the method further comprises administering a tRNA, such as one disclosed hereinabove, that recognizes the codon encoded by the ADAR2 edited sequence. In some

embodiments, the tRNA is a modified endogenous tRNA charged with a canonical amino acid. In some embodiments, the canonical amino acid is serine. In some embodiments, the tRNA is an orthogonal tRNA charged with a non-canonical amino acid. In some embodiments, the orthogonal tRNA has a corresponding synthetase. In some embodiments, the corresponding synthetase is E. coli Glutaminyl-tRNA synthetase. In some embodiments, the non-canonical amino acid is introduced to the subject *(e.g.* through food), allowing for the induction of the orthogonal tRNA activity. In some embodiments, the non-canonical amino acid is pyrrolysine. In some embodiments, the tRNA targets an amber codon. In some embodiments, the tRNA targets an ochre codon. In some embodiments, the tRNA targets an opal codon. In some embodiments, the protein deficiency is a dystrophin deficiency. In some embodiments, the disease, disorder, or condition is muscular dystrophy. In some embodiments, the muscular dystrophy is Duchene muscular dystrophy.

[0017] Other aspects relate to a recombinant expression system comprising one or more vectors encoding an ADAR2 based RNA editing system comprising one or more of an ADAR2, one or more forward guide RNAs for the ADAR2 ("adRNAs"), and one or more corresponding reverse guide RNAs for the ADAR2 ("radRNAs"), wherein the ADAR2 based RNA editing system specifically edits a mutation in an RNA sequence encoding a protein. In some embodiments, the ADAR2 changes adenosine (A) to inosine (I), which is read during translation as guanosine (G). In some embodiments, one adRNA/radRNA pair guides the conversion of UAA to UIA (read as UGA). In further embodiments, a second adRNA/radRNA pair guides the conversion of UIA (read as UGA) to UII (read as UGG). In some embodiments, one adRNA/radRNA pair guides the conversion of UAA to UAI (read as UAG). In some embodiments, the one or more vectors or an additional vector further encodes a tRNA, such as one disclosed hereinabove, that recognizes the codon encoded by the ADAR2 edited sequence. In some embodiments, the tRNA is a modified endogenous tRNA charged with a canonical amino acid. In some embodiments, the canonical amino acid is serine. In some embodiments, the tRNA is an orthogonal tRNA charged with a non-canonical amino acid. In some embodiments, the orthogonal tRNA has a corresponding synthetase. In some embodiments, the corresponding synthetase is E. coli Glutaminyl-tRNA synthetase. In some embodiments, the non-canonical amino acid is introduced to the subject

(e.g. through food), allowing for the induction of the orthogonal tRNA activity. In some embodiments, the non-canonical amino acid is pyrrolysine. In some embodiments, the tRNA targets an amber codon. In some embodiments, the tRNA targets an ochre codon. In some embodiments, the tRNA targets an opal codon. In some embodiments, the protein is dystrophin. In some embodiments, the mutation is a nonsense mutation. In some embodiments, the vector is an Adeno-Associated Virus (AAV) vector. In some embodiments, the AAV vector is an AAV8 vector.

[0018] Additional aspects of this disclosure relate to on-demand, in vivo production of therapeutic proteins, such as, but not limited to, (i) insulin; (ii) neutralizing antibodies for viruses *(e.g.* HIV, HCV, HPV, influenza) and bacteria *(e.g. Staph Aureus;* drug resistant strains). Such method aspects comprise administering to a subject a vector encoding the therapeutic protein with a mutation in its sequence and an ADAR2 based RNA editing system comprising an ADAR2, one or more forward guide RNAs for the ADAR2 ("adRNAs"), and one or more corresponding reverse guide RNAs for the ADAR2 ("radRNAs"), wherein the ADAR2 based RNA editing system specifically edits a mutation in an RNA sequence encoding the protein or one or more vectors encoding said ADAR2, adRNAs, radRNAs. Accordingly, any of the methods and vectors disclosed hereinabove relating to an ADAR2 based RNA editing system specifically edits a mutation in an RNA sequence encoding the protein or a vector encoding one or more vectors encoding said ADAR2, adRNAs, radRNAs.

PARTIAL SEQUENCE LISTING

[0019] mU6, tRNA(U25C) Amber

tcccggggtttccgccaTTTTTTGGTACTGAGtCGCCCaGTCTCAGATAGATCCGACGCCGCC
ATCTCTAGGCCCGCGCCGGCCCCCTCGCACAGACTTGTGGGAGAAGCTCGGCTAC
TCCCCTGCCCCGGTTAATTTGCATATAATATTTCCTAGTAACTATAGAGGCTTAAT
GTGCGATAAAAGACAGATAATCTGTTCTTTTTAATACTAGCTACATTTTACATGA
TAGGCTTGGATTTCTATAAGAGATACAAATACTAAATTATTATTTTAAAAAACAG
CACAAAAGGAAACTCACCCTAACTGTAAAGTAATTGTGTGTTTTGAGACTATAAA
TATCCCTTGGAGAAAAGCCTTGTTTGggaaacctgatcatgtagatcgaaCggactCTAaatccgttcagc
cgggttagattcccggggtttccgccaTTTTTTCCTAGACCCAGCTTTCTTGTACAAAGTTGG

[0020] mU6, tRNA(U25C) Ochre

tcccggggtttccgccaTTTTTTGGTACTGAGtCGCCCaGTCTCAGATAGATCCGACGCCGCC
ATCTCTAGGCCCGCGCCGGCCCCCTCGCACAGACTTGTGGGAGAAGCTCGGCTAC
TCCCCTGCCCCGGTTAATTTGCATATAATATTTCCTAGTAACTATAGAGGCTTAAT
GTGCGATAAAAGACAGATAATCTGTTCTTTTTAATACTAGCTACATTTTACATGA
TAGGCTTGGATTTCTATAAGAGATACAAATACTAAATTATTATTTTAAAAAACAG
CACAAAAGGAAACTCACCCTAACTGTAAAGTAATTGTGTGTTTTGAGACTATAAA
TATCCCTTGGAGAAAAGCCTTGTTTGggaaacctgatcatgtagatcgaaCggactTTAaatccgttcagc
cgggttagattcccggggtttccgccaTTTTTTCCTAGACCCAGCTTTCTTGTACAAAGTTGG

[0021] mU6, tRNA(U25C) Opal

tcccggggtttccgccaTTTTTTGGTACTGAGtCGCCCaGTCTCAGATAGATCCGACGCCGCC
ATCTCTAGGCCCGCGCCGGCCCCCTCGCACAGACTTGTGGGAGAAGCTCGGCTAC
TCCCCTGCCCCGGTTAATTTGCATATAATATTTCCTAGTAACTATAGAGGCTTAAT
GTGCGATAAAAGACAGATAATCTGTTCTTTTTAATACTAGCTACATTTTACATGA
TAGGCTTGGATTTCTATAAGAGATACAAATACTAAATTATTATTTTAAAAAACAG
CACAAAAGGAAACTCACCCTAACTGTAAAGTAATTGTGTGTTTTGAGACTATAAA
TATCCCTTGGAGAAAAGCCTTGTTTGggaaacctgatcatgtagatcgaaCggactTCAaatccgttcagc
cgggttagattcccggggtttccgccaTTTTTTCCTAGACCCAGCTTTCTTGTACAAAGTTGG

[0022] MmPylRS (Aflll)

CAGCCTCCGGACTCTAGAGGATCGAACCCTTAAGgccaccATGGATAAGAAACCTTT
GAACACTCTCATTAGTGCGACAGGGCTCTGGATGTCCCGAACGGGGACTATACA
CAAGATAAAACACCATGAGGTCTCAAGGAGCAAAATCTATATCGAGATGGCATG
CGGCGACCATCTTGTGGTAAATAATAGTAGGTCCTCCAGGACGGCAAGAGCACT
CCGACATCACAAGTACAGAAAAACCTGCAAACGGTGTAGGGTATCCGACGAAGA
CTTGAACAAATTTTTGACTAAGGCCAACGAGGATCAAACTTCTGTCAAAGTGAAA
GTGGTTTCTGCTCCTACCCGAACTAAGAAGGCCATGCCCAAGTCCGTGGCAAGGG
CACCCAAGCCACTCGAAAATACTGAGGCCGCTCAGGCCCAACCATCCGGTAGTA
AGTTCAGTCCAGCCATACCCGTAAGTACCCAAGAATCTGTCAGTGTGCCGGCCTC

AGTTTCCACATCTATAAGTTCAATTTCTACAGGAGCGACGGCCTCCGCCCTCGTC
AAGGGTAACACAAACCCGATAACTTCTATGAGTGCCCCCGTACAGGCATCCGCA
CCAGCACTGACGAAGTCTCAAACTGATAGGCTGGAAGTGCTCTTGAATCCGAAG
GACGAGATATCTCTTAACTCCGGTAAACCTTTCCGGGAGCTGGAAAGTGAACTTC
TCAGCCGGCGAAAAAAAGACCTCCAGCAAATTTACGCAGAGGAAAGGGAGAAC
TATCTGGGGAAGTTGGAACGAGAGATCACCCGATTCTTTGTCGATCGCGGATTTT
TGGAGATTAAAAGCCCAATTCTCATCCCCCTTGAATATATCGAACGAATGGGAAT
CGACAATGATACGGAGTTGTCCAAGCAGATTTTCCGCGTAGACAAGAACTTTTGT
CTTCGACCCATGCTCGCTCCGAACCTCTACAATTACTTGAGAAAGTTGGACAGAG
CGCTCCCGGACCCGATCAAGATATTTGAGATCGGTCCTTGTTATAGAAAGGAGAG
TGATGGAAAAGAACACCTCGAAGAGTTCACGATGCTGAACTTCTGCCAAATGGG
TTCTGGCTGCACACGGGAGAATCTCGAAAGCATCATTACAGATTTCCTTAACCAT
CTGGGGATAGACTTTAAAATAGTGGGTGACAGCTGTATGGTATACGGAGATACC
TTGGACGTAATGCACGGGGATCTTGAGCTTTCCTCCGCCGTGGTTGGACCTATAC
CGTTGGACCGGGAGTGGGGAATCGACAAACCGTGGATAGGCGCCGGTTTCGGCC
TTGAAAGACTCCTCAAAGTCAAGCATGATTTCAAAAACATAAAACGGGCTGCTC
GCTCCGAATCTTATTACAACGGTATAAGTACGAACCTGTGATAATAGCTTAAGGG
TTCGATCCCTACtGGTTAGTAATGAGTTTA

[0023] tRNAs

[0024] Amber suppression:

ggaaacctgatcatgtagatcgaatggactctaaatccgttcagccgggttagattcccggggtttccgcca

[0025] Amber suppression (2):

gggggtggatcgaatagatcacacggactctaaattcgtgcaggcgggtgaaactcccgtactccccgcca

[0026] Ochre supression

ggaaacctgatcatgtagatcgaatggactttaaatccgttcagccgggttagattcccggggtttccgcca

[0027] Opal suppression:

ggaaacctgatcatgtagatcgaatggacttcaaatccgttcagccgggttagattcccggggtttccgcca

[0028] Synthetase:

ATGGATAAAAAACCATTAGATGTTTTAATATCTGCGACCGGGCTCTGGATGTCCA
GGACTGGCACGCTCCACAAAATCAAGCACCATGAGGTCTCAAGAAGTAAAATAT
ACATTGAAATGGCGTGTGGAGACCATCTTGTTGTGAATAATTCCAGGAGTTGTAG
AACAGCCAGAGCATTCAGACATCATAAGTACAGAAAAACCTGCAAACGATGTAG
GGTTTCGGACGAGGATATCAATAATTTTCTCACAAGATCAACCGAAAGCAAAAA
CAGTGTGAAAGTTAGGGTAGTTTCTGCTCCAAAGGTCAAAAAAGCTATGCCGAA
ATCAGTTTCAAGGGCTCCGAAGCCTCTGGAAAATTCTGTTTCTGCAAAGGCATCA
ACGAACACATC::CAGATCTC5IA CCTTXX5CCIXGCAAAATCAACTCX A AATTXX5IXrIX;
TTCCCGCATCGGCTCCTGCTCCTTCACTTACAAGAAGCCAGCTTGATAGGGTTGA
GGCTCTCTTAAGTCCAGAGGATAAAATTTCTCTAAATATGGCAAAGCCTTTCAGG
GAACTTGAGCCTGAACTTGTGACAAGAAGAAAAAACGATTTTCAGCGGCTCTAT
ACCAATGATAGAGAAGACTACCTCGGTAAACTCGAACGTGATATTACGAAATTTT
TCGTAGACCGGGGTTTTCTGGAGATAAAGTCTCCTATCCTTATTCCGGCGGAATA
CGTGGAGAGAATGGGTATTAATAATGATACTGAACTTTCAAAACAGATCTTCCGG
GTGGATAAAAATCTCTGCTTGAGGCCAATGCT TGCCCCGACTCTTTACAACTATC
TGCGAAAACTCGATAGGATTTTACCAGGCCCAATAAAAATTTTCGAAGTCGGACC
TTGTTACCGGAAAGAGTCTGACGGCAAAGAGCACCTGGAAGAATTTACTAT GGT
GAACTTCTGTCAGATGGGTTCGGGATGTACTCGGGAAAATCTTGAAGCTCTCATC
AAAGAGTTTCTGGACT ATCTGGAAATCGACTTCGAAATCGTAGGAGATTCCTGTA
TGGTCTTTGGGGATACTCTTGATATAATGCACGGGGACCTGGAGCTTTCTTCGGC
AGTCGTCGGGCCAGTTTCTCTTGATAGAGAATGGGGTATTGACAAACCATGGATA
GGTGCAGGTTTTGGTCTTGAACGCTTGCTCAAGGTTATGCACGGCTTTAAAAACA
TTAAGAGGGCATCAAGGTCCGAATCTTACTATAATGGGATTTCAACCAATCTGTA
A

[0029] EGFP:

atggtgagcaagggcgaggagctgttcaccggggtggtgcccatcctggtcgagctggacggcgacgtaaacggccacaagttca
gcgtgtccggcgagggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgcaccaccggcaagctgcccgtgcc
ctggcccaccctcgtgaccaccctgacctacggcgtgcagtgcttcagccgctaccccgaccacatgaagcagcacgacttcttcaa
gtccgccatgcccgaaggctacgtccaggagcgcaccatcttcttcaaggacgacggcaactacaagacccgcgccgaggtgaagt
tcgagggcgacaccctggtgaaccgcatcgagctgaagggcatcgacttcaaggaggacggcaacatcctggggcacaagctgga
gtacaactacaacagccacaacgtctatatcatggccgacaagcagaagaacggcatcaaggtgaacttcaagatccgccacaacat
cgaggacggcagcgtgcagctcgccgaccactaccagcagaacaccccatcggcgacggccccgtgctgctgcccgacaacca
ctacctgagcacccagtccgccctgagcaaagaccccaacgagaagcgcgatcacatggtcctgctggagttcgtgaccgccgccg
ggatcactctcggcatggacgagctgtacaagtaa

[0030] EGFP Amber:

Atggtgagcaagggcgaggagctgttcaccggggtggtgcccatcctggtcgagctggacggcgacgtaaacggccacaagttca
gcgtgtccggcgagggcgagggcgatgccacctagggcaagctgaccctgaagttcatctgcaccaccggcaagctgcccgtgcc
ctggcccaccctcgtgaccaccctgacctacggcgtgcagtgcttcagccgctaccccgaccacatgaagcagcacgacttcttcaa
gtccgccatgcccgaaggctacgtccaggagcgcaccatcttcttcaaggacgacggcaactacaagacccgcgccgaggtgaagt
tcgagggcgacaccctggtgaaccgcatcgagctgaagggcatcgacttcaaggaggacggcaacatcctggggcacaagctgga
gtacaactacaacagccacaacgtctatatcatggccgacaagcagaagaacggcatcaaggtgaacttcaagatccgccacaacat
cgaggacggcagcgtgcagctcgccgaccactaccagcagaacaccccatcggcgacggccccgtgctgctgcccgacaacca
ctacctgagcacccagtccgccctgagcaaagaccccaacgagaagcgcgatcacatggtcctgctggagttcgtgaccgccgccg
ggatcactctcggcatggacgagctgtacaagtaatga

[0031] EGFP Ochre:

atggtgagcaagggcgaggagctgttcaccggggtggtgcccatcctggtcgagctggacggcgacgtaaacggccacaagttca
gcgtgtccggcgagggcgagggcgatgccacctaaggcaagctgaccctgaagttcatctgcaccaccggcaagctgcccgtgcc
ctggcccaccctcgtgaccaccctgacctacggcgtgcagtgcttcagccgctaccccgaccacatgaagcagcacgacttcttcaa
gtccgccatgcccgaaggctacgtccaggagcgcaccatctictttcaaggacgacggcaactacaagacccgcgccgaggigaagt
tcgagggcgacaccctggtgaaccgcatcgagctgaagggcatcgacttcaaggaggacggcaacatcctggggcacaagctgga
gtacaactacaacagccacaacgictaiatcatggccgacaagcagaagaacggcaicaaggtgaacttcaagatccgccacaacai
cgaggacggcagcgtgcagctcgccgaccactaccagcagaacaccccatcggcgacggccccgtgctgctgcccgacaacca

ctacctgagcacccagtccgccctgagcaaagaccccaacgagaagcgcgatcacatggtcctgctggagttcgtgaccgccgccg
ggatcactctcggcatggacgagctgtacaagtaatga

[0032] EGFP Opal:

Atggtgagcaagggcgaggagctgttcaccggggtggtgcccatcctggtcgagctggacggcgacgtaaacggccacaagttca
gcgtgtccggcgagggcgagggcgatgccacctgaggcaagctgaccctgaagttcatctgcaccaccggcaagctgcccgtgcc
ctggcccaccctcgtgaccaccctgacctacggcgtgcagtgcttcagccgctaccccgaccacatgaagcagcacgacttcttcaa
gtccgccatgcccgaaggctacgtccaggagcgcaccatcttcttcaaggacgacggcaactacaagacccgcgccgaggtgaagt
tcgagggcgacaccctggtgaaccgcatcgagctgaagggcatcgacttcaaggaggacggcaacatcctggggcacaagctgga
gtacaactacaacagccacaacgtctatatcatggccgacaagcagaagaacggcatcaaggtgaacttcaagatccgccacaacat
cgaggacggcagcgtgcagctcgccgaccactaccagcagaacaccccatcggcgacggccccgtgctgctgcccgacaacca
ctaccigagcacccagtccgccctgagcaaagaccccaacgagaagcgcgatcacatggtcctgctggagttcgtgaccgccgccg
ggatcactctcggcatggacgagctgtacaagtaatga

[0033] MbPylRS

10 20 30 40 50
MDKKPLDVLI SATGLWMSRT GTLHKIKHHE VSRSKIYIEM ACGDHLWNN
60 70 80 90 100
SRSCRTARAF RHHKYRKTCK RCRVSDEDIN NFLTRSTESK NSVKVRVVSA
110 120 130 140 150
PKVKKAMPKS VSRAPKPLEN SVSAKASTNT SRSVPSPAKS TPNSSVPASA
160 170 180 190 200
PAPSLTRSQL DRVEALLSPE DKISLNMAKP FRELEPELVT RRKNDFQRLY
210 220 230 240 250
TNDREDYLGK LERDITKFFV DRGFLEIKSP ILIPAEYVER MGINKDTELS
260 270 280 290 300
KQIFRVDKNL CLRPMLAPTL YNYLRKLDRI LPGPIKIFEV GPCYRKESDG
310 320 330 340 350
KEHLEEFTMV NFCQMGSGCT RENLEALIKE FLDYLEIDFE IVGDSCMVYG
360 370 380 390 400
DTLDIMHGDL ELSSAVVGPV SLDREWGIDK PWIGAGFGLE RLLKVMHGFK
410
NIKRASRSES YYNGISTNL

[0034] MmPylRS (uniprot)

10 20 30 40 50
MDKKPLNTLI SATGLWMSRT GTIHKIKHHE VSRSKIYTEM ACGDHLVVNN
60 70 80 90 100
SRSSRTARAL RHHKYRKTCK RCRVSDEDLN KFLTKANEDQ TSVKVKWSA
110 120 130 140 150
PTRTKKAMPK SVARAPKPLE NTEAAQAQPS GSKF8PAIPV STQESVSVPA
160 170 180 190 200
SVSTSiSSiS TGATASALVK GNTNPITSMS APVQASAPAL TKSQTDRLEV
210 220 230 240 250
LLNPKDEISL NSGKPFRELE SELLSRRKKD LQQIYAEERE NYLGKLEREI
260 270 280 290 300
TRFFVDRGFL EIKSPILIPL EYIERMGIDN DTELSKQIFR VDKNFCLRPM
310 320 330 340 350
LAPNLYNYLR KLDRALPDPI KIFEIGPCYR KESDGKEHLE EFTMLNFCQM
360 370 380 390 400
GSGCTRENLE SIITDFLNHL G1DFKIVGDS CMVYGDTLDV MHGDLELSSA
410 420 430 440 450
VVGPIPLDRE WGIDKPWIGA GFGLERLLKV KHDFKNIKRA ARSESYYNGI

STNL

[0035] PylT* (Amber)

[0036]

ggaaacctgatcatgtagatcgaaCggactCTAaatccgttcagccgggttagattcccggggtttccgccaTTTTTT

[0037] PylT* (Ochre)

[0038]

ggaaacctgatcatgtagatcgaaCggactTTAaatccgttcagccgggttagattcccggggtttccgccaTTTTTT

[0039] PylT* (Opal)

[0040]

ggaaacctgatcatgtagatcgaaCggactTCAaatccgttcagccgggttagattcccggggtttccgccaTTTTTT

[0041] Mouse U6 primers

[0042] tcccggggtttccgccaTTTTTTGGTACTGAGtCGCCCaGTCTCAGAT

[0043] CAAACAAGGCTTTTCTCCAAGGGATAT

[0044] tRNA (U25C) Amber_F:
CCTTGGAGAAAAGCCTTGTTTGggaaacctgatcatgtagatcgaacggactCTAaatccgttcagccggg

[0045] Common reverse:

[0046] PylT

[0047] ggaaacctgatcatgtagatcgaatggactCTAaatccgttcagccgggttagattcccggggtttccgcca

[0048] PylT*(U25C)

[0049] ggaaacctgatcatgtagatcgaaCggactCTAaatccgttcagccgggttagattcccggggtttccgcca

1. Arg tRNA (opal) (E-Cadherin paper)
GGCCGCGTGGCCTAATGGATAAGGCGTCTGACTJC4GATCAGAAGATTGCAG
GTTCGAGTCCTGCCGCGGTCG

2. Arg tRNA (opal) (Xeroderma paper)
GACCACGTGGCCTAATGGATAAGGCGTCTGACTrC4GATCAGAAGATTGAGG
GTTCGAATCCCTTCGTGGTTA

3. Serine tRNA (amber)
GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTC7MAATCCATTGGGGTTTCC
CCGCGCAGGTTCGAATCCTGCCGACTACG

4. Leucine tRNA (amber)
GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTCTMGTTCTGGTCTCCAATG
GAGGCGTGGGTTCGAATCCCACTTCTGACA

[0050] Forward:

[0051] **TTGTGGAAAGGACGAAACACC**

[0052] Reverse:

[0053] ACAAGAAAGCTGGGTCTAGGCTAGCAAAAAA

[0054] tRNA_Leu_Am_F (overlaps with vector, **bold;** anti-codon sequences, **bold underline**):

TTGTGGAAAGGACGAAACACCGGTCAGGATGGCCGAGTGGTCTAAGGCGCCAG
ACTC7MGTTCTGGTCTCCAATGG

[0055] tRNA_Leu_Oc_F (overlaps with vector, **bold;** anti-codon sequences, **bold underline) :**

TTGTGGAAAGGACGAAACACCGGTCAGGATGGCCGAGTGGTCTAAGGCGCCAG
ACT7X4GTTCTGGTCTCCAATGG

[0056] tRNA_Leu_Op_F (overlaps with vector, **bold;** anti-codon sequences, **bold underline) :**

TTGTGGAAAGGACGAAACACCGGTCAGGATGGCCGAGTGGTCTAAGGCGCCAG
ACTJC4GTTCTGGTCTCCAATGG

[0057] tRNA _Leu_R (overlaps with vector, **bold;** anti-codon sequences, **bold underline) :**

ACAAGAAAGCTGGGTCTAGGCTAGCAAAAAATGTCAGAAGTGGGATTCGAAC
CCACGCCTCCATTGGAGACCAGAAC

[0058] tRNA_Ser_Am_F (overlaps with vector, **bold;** anti-codon sequences, **bold underline) :**

TTGTGGAAAGGACGAAACACCGGTAGTCGTGGCCGAGTGGTTAAGGCGATGGA
CTC7MAATCCATTGGGGTTTCC

[0059] tRNA_Ser_Oc_F (overlaps with vector, **bold;** anti-codon sequences, **bold underline) :**

TTGTGGAAAGGACGAAACACCGGTAGTCGTGGCCGAGTGGTTAAGGCGATGGA
CTT7¾AATCCATTGGGGTTTCC

[0060] tRNA_Ser_Op_ (overlaps with vector, **bold;** anti-codon sequences, **bold underlined :**

TTGTGGAAAGGACGAAACACCGGTAGTCGTGGCCGAGTGGTTAAGGCGATGGA
CTrC4AATCCATTGGGGTTTCC

[0061] tRNA _Ser_R (overlaps with vector, **bold;** anti-codon sequences, **bold underline) :**

ACAAGAAAGCTGGGTCTAGGCTAGCAAAAAACGTAGTCGGCAGGATTCGAAC
CTGCGCGGGGAAACCCCAATGGATT

[0062] tRNA_Arg_Am_F (overlaps with vector, **bold;** anti-codon sequences, **bold underline**):

TTGTGGAAAGGACGAAACACCGGACCACGTGGCCTAATGGATAAGGCGTCTGA
CTrC4GATCAGAAGATTGAGGGTT

[0063] tRNA_Arg_Oc_F (overlaps with vector, **bold;** anti-codon sequences, **bold underline) :**

TTGTGGAAAGGACGAAACACCGGACCACGTGGCCTAATGGATAAGGCGTCTGA
CT7X4GATCAGAAGATTGAGGGTT

[0064] tRNA_Arg_Op_F (overlaps with vector, **bold;** anti-codon sequences, **bold underline) :**

TTGTGGAAAGGACGAAACACCGGACCACGTGGCCTAATGGATAAGGCGTCTGA
CTrC4GATCAGAAGATTGAGGGTT

[0065] tRNA _Arg_R (overlaps with vector, **bold;** anti-codon sequences, **bold underline**):

ACAAGAAAGCTGGGTCTAGGCTAGCAAAAAATAACCACGAAGGGATTCGAAC
CCTCAATCTTCTGATC

[0066] mU6_tRNA_ser_oc :
GTACTGAGtCGCCCaGTCTCAGATAGATCCGACGCCGCCATCTCTAGGCCCGCGCC

GGCCCCCTCGCACAGACTTGTGGGAGAAGCTCGGCTACTCCCCTGCCCCGGTTAA
TTTGCATATAATATTTCCTAGTAACTATAGAGGCTTAATGTGCGATAAAAGACAG
ATAATCTGTTCTTTTTAATACTAGCTACATTTTACATGATAGGCTTGGATTTCTAT
AAGAGATACAAATACTAAATTATTATTTTAAAAAACAGCACAAAAGGAAACTCA
CCCTAACTGTAAAGTAATTGTGTGTTTTGAGACTATAAATATCCCTTGGAGAAAA
GCCTTGTTTGGTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTTTAAATCCATTG
GGGTTTCCCCGCGCAGGTTCGAATCCTGCCGACTACGTTTTTT

[0067] mU6_tRNA_ser_oc_Nhe 1_insert_F :

[0068] AATCCTGCCGACTACGTTTTTTGTACTGAGtCGCCCAGTCT

[0069] adRNA (premature stop codon target, **bold;** edited bases, **bold underline) :**

Sequential edits:
TTTGAAAGAGCAATAAAAT
CTTTGAAAGAGCAATAGAA
Dual edits:
TTTGAAAGAGCAATAAAAT

[0070] radRNA (premature stop codon target, **bold;** edited bases, **bold underline) :**

Sequential edits:
Ataa AATGGC TTCAACTAT
AAtagAATGGCTTCAACTA
Dual edits:
AAtaaAATGGCTTCAACTA

[0071] OTC target (edited bases, **bold):**

[0072] TCACAGACACCGCTCAGTTTGT

[0073] Optimization of the length of adRNA and distance of the edit from the ADAR2 recruiting domain (Length of adRNA - distance of edit from ADAR2 recruiting domain):

16-5: atgccaccTGGggcaa

16-6: tgccaccTGGggcaag

16-7: gccaccTGGggcaagc

18-6: gatgccaccTGGggcaag

20-6: gcgatgccaccTGGggcaag

[0074] ADAR2 recruiting region v1:

[0075] GGGTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACCT

[0076] ADAR2 recruiting region v2:

[0077] GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCAC

[0078] Hairpin (3') **(FIG. 8):** GGGCCCTCTTCAGGGCCCTCTAGA

[0079] Hairpin (3') **(FIG. 10):** atcgccctgaaaag

[0080] Toe hold (5'): gccaccTGGgg

[0081] List of suppressor tRNA sequences:

Suppressor tRNAs	**Sequence (5' to 3')**
Serine	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTNNNAATCCATTGG GGTTTCCCCGCGCAGGTTCGAATCCTGCCGACTACG
Leucine	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTNNNGTTCTGGTC TCCAATGGAGGCGTGGGTTCGAATCCCACTTCTGACA
Arginine	GACCACGTGGCCTAATGGATAAGGCGTCTGACTNNNGATCAGAAG ATTGAGGGTTCGAATCCCTTCGTGGTTA

[0082] NNN – anticodon

In endogenous tRNA, the tRNA is modified to recognize the codon comprising the point mutation by including the complementary sequence at the NNN position noted herein above. As clarified in more detail below, the NNN sequences in amber, ochre, and opal tRNA are as follows: Amber: NNN=CTA; Ochre: NNN=TCA; Opal: NNN=TTA.

[0083] List of primers for next generation sequencing (NGS) analyses.

Name	Sequence (5' to 3')
NGS_DMD_F1	GTGTTACTGAATATGAAATAATGGAGGA
NGS_DMD_R1	ATTTCTGGCATATTTCTGAAGGTG
NGS_DMD_F2	CTCTCTGTACCTTATCTTAGTGTTACTGA
NGS_DMD_R2	CTCTTCAAATTCTGACAGATATTTCTGGC
NGS_OTC_F	ACCCTTCCTTTCTTACCACACA
NGS_OTC_R	CAGGGTGTCCAGATCTGATTGTT
NGS_OTC_R2	CTTCTCTTTTAAACTAACCCATCAGAGTT

[0084] List of adRNA antisense sequences and corresponding ADAR2 recruiting scaffold used for *in vivo* RNA editing studies. In some embodiments, the recruiting scaffold v2 - disclosed in paragraph [0083], is used with these sequences.

Name	**adRNA antisense sequence (3' to 5')**
OTC	**TGTCTGTGGCGAGCCAAACA**
DMD	**ACTTTCTCGTTACCTTACCG**

[0085] **MCP**-*J inke*r-ADARl -NLS (optional sequence in brackets)

MASNFTQFVLVDNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSSA QNRKYTIKVEVPKGAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPS AIAANSGIYGGSGSGAGSGSiMGGG *APGS*GGG^AERMGFTEVTPVTGASLRRTML LLSRSPEAQPKTLPLTGSTFHDQIAMLSHRCFNTLTNSFQPSLLGRKILAAIIMKKDSE DMGVVVSLGTGNRCVKGDSLSLKGETVNDCHAEIISRRGFIRFLYSELMKYNSQTAK

DSIFEPAKGGEKLOIKKTVSFHLYISTAPCGDGALFDKSCSDRAMESTESRHYPVFEN
PKQGKLRTKVENGEGTIPVESSDIVPTWDGIRLGERLRTMSCSDKILRWNVLGLQGA
LLTHFLQPIYLKSVTLGYLFSQGHLTRAICCRVTRDGSAFEDGLRHPFIVMiPKVGRV
SIYDSKRQSGKTKETSVNWCLADGYDLEILDGTRGTVDGPRNELSRVSKKNIFLLFK
KLCSFRYRRDLLRLSYGEAKKAARDYETAKNYFKKGLKDMGYGNWISKPQEEKNF
YLCPVGSGSGSGPKKRKV[AA]*

[0086] *MCY-Linker-*ADAR2 (optional sequence in brackets)

**MGPKKKRKVAAGSGSGSMASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISSN
SRSQAYKVTCSVRQSSAQKRKYTIKVEVPKVATQTVGGVELPVAAWRSYLNME
LTIPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANSGIYGG *SGGSGGS*MLHLDQTP**
SRQPgSEGLQLHLPQVLADAVSRLVLGKFGDLTDNFSSPHARRKVLAGVVMTTGTD
VKDAKVISVSTGTKCINGEYMSDRGLALNDCHAEIISRRSLLRFLYTQLELYLNNKDD
QKRSIFQKSERGGFRLKENVQFHLYISTSPCGDARIFSPHEPILEEPADRHPNRKARGQ
LRTKIESGEGTIPVRSNASIQTWDGVLQGERLLTMSCSDKIARWNVVGIQGSLLSIFVE
PIYFSSIILGSLYHGDHLSRAMYQRISNIEDLPPLYTLNKPLLSGISNAEARQPGKAPNF
SVNWTVGDSAIEVINATTGKDELGRASRLCKHALYCRWMRVHGKVPSHLLRSKITK
PNVYHESKLAAKEYQAAKARLFTAFIKAGLGAWVEKPTEQDQFSLTrPl*

[0087] **N22p-***Jinke*r-ADAR1-NLS (optional sequence in brackets)

MGNARTRRRERRAEKQAQWKAANGGGGTSGSGSGSiMGGG *APGSGGGSKAER*
MGFTEVTPVTGASLRRTMLLLSRSPEAQPKTLPLTGSTFHDQIAMLSHRCFNTLTNSF
QPSLLGRKILAAIIMKKDSEDMGVVVSLGTGNRCVKGDSLSLKGETVNDCHAEIISRR
GFIRFLYSELMKYNSQTAKDSIFEPAKGGEKLQIKKTVSFHLYISTAPCGDGALFDKS
CSDRAMESTESRHYPVFENPKQGKLRTKVENGEGTIPVESSDIVPTWDGIRLGERLRT
MSCSDKILRWNVLGLQGALLTHFLQPIYLKSVTLGYLFSQGHLTRAICCRVTRDGSA
FEDGLRHPFIVMIPKVGRVSIYDSKROSGKTKETSVNWCLADGYDLEILDGTRGTVD
GPRNELSRVSKKNIFLLFKKLCSFRYRRDLLRLSYGEAKKAARDYETAKNYFKKGLK
DMGYGNWISKPQEEKNFYLCPVGSGSGSGPKKRKVrAAl *

[0088] Nuclear Localization Sequence-*Jinke*r-**N22p**-*Jinke*r-ADAR2 (optional sequence in brackets)

[**MG**]**PKKKRKVAAG^G5MGNARTRRRERRAEKQAQWKAANGGGGTSGSGSG**
*SPAGGGAPGSGGGSM*LHL*DQ*TPSRQPIPSEGLQLHLPQVLADAVSRLVLGKFGDLTD
NFSSPHARRKVLAGVVMTTGTDVKDAKVISVSTGTKCINGEYMSDRGLALNDCHAE
IISRRSLLRFLYTQLELYLNNKDDQKRSIFQKSERGGFRLKENVQFHLYISTSPCGDAR
IFSPHEPILEEPADRHPNRKARGQLRTKIESGEGTIPVRSNASIQTWDGVLQGERLLTM
SCSDKIARWNVVGIQGSLLSIFVEPIYFSSIILGSLYHGDHLSRAMYQRISNIEDLPPLY
TLNKPLLSGISNAEARQPGKAPNFSVNWTVGDSAIEVINATTGKDELGRASRLCKHA
LYCRWMRVHGKVPSHLLRSKITKPNVYHESKLAAKEYQAAKARLFTAFIKAGLGA
WVEKPTEQDQFSLTrPl*

[0089] **MCP**-*Jinke*r-ADARl **(E1008OVNLS** (optional sequence in brackets)

MASNFTQFVLVDNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSSA QNRKYTIKVEVPKGAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPS AIAANSGIYGGSGSGAGSGSiMGGG *AP*GS*S*GGGSKAERMGFTEVTPVTGASLRRTML LLSRSPEAOPKTLPLTGSTFHDOIAMLSHRCFNTLTNSFQPSLLGRKILAAIIMKKDSE DMGVVVSLGTGNRCVKGDSLSLKGETVKDCHAEIISRRGFIRFLYSELMKYNSQTAK DSIFEPAKGGEKLQIKKTVSFHLYISTAPCGDGALFDKSCSDRAMESTESRHYPVFEN PKQGKLRTKVENGOGTIPVES SDIVPTWDGIRLGERLRTMSC SDKILRWNVLGLQGA LLTHFLQPIYLKSVTLGYLFSQGHLTRAICCRVTRDGSAFEDGLRHPFIVMiPKVGRV SIYDSKRQSGKTKETSVNWCLADGYDLEILDGTRGTVDGPRNELSRVSKKNIFLLFK KLCSFRYRRDLLRLSYGEAKKAARDYETAKNYFKKGLKDMGYGNWISKPQEEKNF YLCPVGSGSGSGPKKRKV[AA]*

[0090] Nuclear Localization *S*eque*nc*e-*Linker-MC*P-*Linker-ADAR2 (***E488Q***) (optional sequence in brackets)

[**MG**]**PKKKRKVAAG^G5MASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISS NSRSQAYKVTCSVRQSSAQKRKYTIKVEVPKVATQTVGGVELPVAAWRSYLNM ELTIPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANSGIYGG** *S***GG***S***GG***S**MLHLDQT PSRQPIPSEGLQLHLPQVLADAVSRLVLGKFGDLTDNFSSPHARRKVLAGVVMTTGT DVKDAKVISVSTGTKCINGEYMSDRGLALNDCHAEIISRRSLLRFLYTQLELYLNNKD DQKRSIFQKSERGGFRLKENVQFHLYISTSPCGDARIFSPHEPILEEPADRHPNRKARG QLRTKIESGOGTIPVRSNASIQTWDGVLOGERLLTMSCSDKIARWNVVGIQGSLLSIF VEPIYFSSIILGSLYHGDHLSRAMYQRISNIEDLPPLYTLNKPLLSGISNAEARQPGKAP NFSVNWTVGDSAIEVINATTGKDELGRASRLCKHAL YCRWMRVHGKVP SFILLRSKI TKPNVYHESKLAAKEYQAAKARLFTAFIKAGLGAWVEKPTEQDQF SLTrPl*

[0091] **N22p**-*Jin*ke r-ADAR1 **(E1008Q)** (optional sequence in brackets)

MGNARTRRRERRAEKQAQWKAANGGGGTSGSGSGSiMGGG *AP***G***S***GGG***S**KAER MGFTEVTPVTGASLRRTMLLLSRSPEAQPKTLPLTGSTFHDQIAMLSHRCFNTLTNSF QPSLLGRKILAAIIMKKDSEDMGVVVSLGTGNRCVKGDSLSLKGETVNDCHAEIISRR GFIRFLYSELMKYNSQTAKDSIFEPAKGGEKLQIKKTVSFHLYISTAPCGDGALFDKS CSDRAMESTESRHYPVFENPKQGKLRTKVENGOGTIPVESSDIVPTWDGIRLGERLRT MSCSDKILRWNVLGLQGALLTHFLQPIYLKSVTLGYLFSQGHLTRAICCRVTRDGSA FEDGLRHPFIVNFiPKVGRVSIYDSKROSGKTKETSVNWCLADGYDLEILDGTRGTVD GPRNELSRVSKKNIFLLFKKLCSFRYRRDLLRLSYGEAKKAARDYETAKNYFKKGLK DMGYGNWISKPQEEKNFYLCPVGSGSGSGPKKRKVrAAl *

[0092] Nuclear Localization Sequence-*Jin*ke r-**N22p**-*Jin*ke r-ADAR2 **(E488Q)**

[**MG**]**PKKKRKVAAG^G5MGNARTRRRERRAEKQAQWKAANGGGGTSGSGSG** *SPAGGGAPGSGGGSMLHLDQ*TPSRQPIPSEGLQLHLPQVLADAVSRLVLGKFGDLTD NFSSPHARRKVLAGVVMTTGTDVKDAKVISVSTGTKCINGEYMSDRGL ALNDCHAE IISRRSLLRFLYTQLELYLNNKDDQKRSIFQKSERGGFRLKENVQFHLYISTSPCGDAR IFSPHEPILEEPADRHPNRKARGQLRTKIESGOGTIPVRSNASIQTWDGVLQGERLLTM

SCSDKIARWNVVGIQGSLLSIFVEPIYFSSIILGSLYHGDHLSRAMYQRISNIEDLPPLY
TLNKPLLSGISNAEARQPGKAPNFSVNWTVGDSAIEVINATTGKDELGRASRLCKHA
LYCRWMRVHGKVPSHLLRSKITKPNVYHESKLAAKEYQAAKARLFTAFIKAGLGA
WVEKPTEQDQFSLTrPl*

[0093] Nuclear Localization Sequence-*Linker*-**MCP**-*Linker*-hAPOPECl

[**MG**]**PKKKRKVAAG^G5MASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISS NSRSQAYKVTCSVRQSSAQKRKYTIKVEVPKVATQTVGGVELPVAAWRSYLNM ELTIPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANSGIYGG** *S***GG***S***GG***S**MTSEKGP
STGDPTLRRRIEPWEFDVFYDPRELRKEACLLYEIKWGMSRKIWRSSGKNTTNHVEV
NFIKKFT SERDFFiP SMSC SITWFL SWSPCWEC SOAIREFL SRFiPGVTLVIYVARLFWH
MDQONRQGLRDLVNSGVTIQIMRASEYYHCWRNFVNYPPGDEAHWPQYPPLWMM
LYALELHCIILSLPPCLKISRRWQNHLTFFRLHLQNCHYQTIPPHILLATGLIHPSVAWR
*
–

[0094] Nuclear Localization Sequence-*Jinke*r-**MCP**-*Jinke*r-rAPOBECl

[**MG**]**PKKKRKVAAG^G5MASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISS NSRSQAYKVTCSVRQSSAQKRKYTIKVEVPKVATQTVGGVELPVAAWRSYLNM ELTIPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANSGIYGG** *S***GG***S***GG***S**MSSETGP
VAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEV
NFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHAD
PRNRQGLRDLISSGVTIQFMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLE
LYCIILGLPPCLNILRRKOPOLTFFTIALOSCHYORLPPHILWATGLK*

[0095] **dsRBD**-*Jinke*r-rAPOBEC**1**

MDIEDEENMSSSSTDVKENRNLDNVSPKDGSTPGPGEGSQLSNGGGGGPGRKRP LEEGSNGHSKYRLKKRRKTPGPVLPKNALMQLNEIKPGLQYTLLSQTGPVHAP LFVMSVEVNGQVFEGSGPTKKKAKLHAAEKALRSFVQFPNASEAHLAMGRTLS VNTDFTSDQADFPDTLFNGFETPDKAEPPFYVGSNGDDSFSSSGDLSLSASPVPAS LAQPPLPVLPPFPPPSGKNPVMILNELRPGLKYDFLSESGESHAKSFVMSVVVDG QFFEGSGRNKKLAKARAAQSALAAIFNGG *S***GG***S***GG***S**MSSETGPVAVDPTLRRRIEP
HEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVNFIEKFTTERYFCP
NTRC SITWFL SW SPC GEC SR AITEFL SRYPHVTLFIYIARL YHHADPRNRQGLRDLI SS
GVTIQIMTEQESGYCWRNFWYSPSNEAHWPRYPHLWVRLYVLELYCIILGLPPCLNI
LRRKOPOLTFFTIALOSCHYORLPPHILWATGLK*

[0096] **dsRBD**-*Jinke*r-hAPOBECl

MDIEDEENMSSSSTDVKENRNLDNVSPKDGSTPGPGEGSQLSNGGGGGPGRKRP LEEGSNGHSKYRLKKRRKTPGPVLPKNALMQLNEIKPGLQYTLLSQTGPVHAP LFVMSVEVNGQVFEGSGPTKKKAKLHAAEKALRSFVQFPNASEAHLAMGRTLS VNTDFTSDQADFPDTLFNGFETPDKAEPPFYVGSNGDDSFSSSGDLSLSASPVPAS LAQPPLPVLPPFPPPSGKNPVMILNELRPGLKYDFLSESGESHAKSFVMSVVVDG QFFEGSGRNKKLAKARAAQSALAAIFNGG *S***GG***S***GG***S**MTSEKGPSTGDPTLRRRIEP

WEFDVFYDPRELRKEACLLYEIKWGMSRKIWRSSGKNTTNHVEVNFIKKFTSERDFH
PSMSCSITWFLSWSPCWECSQAIREFLSRHPGVTLVIYVARLFWHMDQQNRQGLRDL
WSGVTIQIMRASEYYHCWRNFVNYPPGDEAHWPQYPPLWMMLYALELHCIILSLP
PCLKISRRWQNHLTFFRLHLQNCHYQTIPPHILLATGLIHPSVAWR*

[0097] **MCP-*J inke*r-ADARl** -NES

**MASNFTQFVLVDNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSSA
QNRKYTIKVEVPKGAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPS
AIAANSGIYGGSGSGAGSGSiMGGG** *AP*G*S*GGG^AERMGFTEVTPVTGASLRRTML
LLSRSPEAQPKTLPLTGSTFHDQIAMLSHRCFNTLTNSFQPSLLGRKILAAIIMKKDSE
DMGVVVSLGTGNRCVKGDSLSLKGETVKDCHAEIISRRGFIRFLYSELMKYNSQTAK
DSIFEPAKGGEKLQIKKTVSFHLYISTAPCGDGALFDKSCSDRAMESTESRHYPVFEN
PKQGKLRTKVENGEGTIPVESSDIVPTWDGIRLGERLRTMSCSDKILRWNVLGLQGA
LLTHFLQPIYLKSVTLGYLFSQGHLTRAICCRVTRDGSAFEDGLRHPFIVMiPKVGRV
SIYDSKROSGKTKETSVNWCLADGYDLEILDGTRGTVDGPRNELSRVSKKNIFLLFK
KLCSFRYRRDLLRLSYGEAKKAARDYETAKNYFKKGLKDMGYGNWISKPQEEKNF
YLCPVGSGSGSLPPLERLTL*

[0098] **MCP-*J inke*r-**ADAR2-NLS

**MASNFTQFVLVDNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSSA
QNRKYTIKVEVPKGAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPS
AIAANSGIYGGSGSGAGSGSiMGGG** *AP*G*S*GGG^QLHLPQVLADAVSRLVLGKFGDL
TDNFSSPHARRKVLAGVVMTTGTDVKDAKVISVSTGTKCINGEYMSDRGLALNDCH
AEIISRRSLLRFLYTQLELYLNNKDDQKRSIFQKSERGGFRLKENVQFHLYISTSPCGD
ARIFSPHEPILEEPADRHPNRKARGQLRTKIESGEGTIPVRSNASIQTWDGVLQGERLL
TMSCSDKIARWNVVGIQGSLLSIFVEPIYFSSIILGSLYHGDHLSRAMYQRISNIEDLPP
LYTLNKPLLSGISNAEARQPGKAPNFSVNWTVGDSAIEVINATTGKDELGRASRLCK
HALYCRWMRVHGKVPSHLLRSKITKPNVYHESKLAAKEYQAAKARLFTAFIKAGLG
AWVEKPTEQDQFSLTGSGSGSPKKKRKV*

[0099] **MCP-*J inke*r-**ADAR2-NES

**MASNFTQFVLVDNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSSA
QNRKYTIKVEVPKGAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPS
AIAANSGIYGGSGSGAGSGSiMGGG** *AP*G*S*GGG^QLHLPQVLADAVSRLVLGKFGDL
TDNFSSPHARRKVLAGVVMTTGTDVKDAKVISVSTGTKCINGEYMSDRGLALNDCH
AEIISRRSLLRFLYTQLELYLNNKDDQKRSIFQKSERGGFRLKENVQFHLYISTSPCGD
ARIFSPHEPILEEPADRHPNRKARGQLRTKIESGEGTIPVRSNASIQTWDGVLQGERLL
TMSCSDKIARWNVVGIQGSLLSIFVEPIYFSSIILGSLYHGDHLSRAMYQRISNIEDLPP
LYTLNKPLLSGISNAEARQPGKAPNFSWWTVGDSAIE VINATTGKDELGRASRLCK
HALYCRWMRVHGKVPSHLLRSKITKPNVYHESKLAAKEYQAAKARLFTAFIKAGLG
AWVEKPTEODQFSLTGSGSGSLPPLERLTL*

[0100] **MCP-***Jinke*r-rAPOBEC 1-NLS

MASNFTQFVLVDNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSSA QNRKYTIKVEVPKGAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPS AIAANSGIYGGSGSGAGSGSiM *GGGAPGSGGGSSGSETPGTSESATPESM*SSETGPV*A* VDPTLRRRIEPHEFEWFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVKFI EKFT TERYFCPNTRC SITWFL SWSPCGEC SRAITEFL SRYPHVTLFIYIARLYF1HADPRNRQG LRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLELYCIILG LPPCLNILRRKOPOLTFFTIALOSCHYQRLPPHILWATGLKGSGSGSPKKKRKV *

[0101] **MCP-***Jinke* **r-**rAPOBEC 1-NE S

MASNFTQFVLVDNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSSA QNRKYTIKVEVPKGAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPS AIAANSGIYGGSGSGAGSGSiM *GGGAPGSGGGSSGSETPGTSESATPESM*SSETGPV*A* VDPTLRRRIEPHEFEWFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVNFI EKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHADPR **NROGLRDLISSGVTIOIMTEOESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLEL** YCnLGLPPCLNILRRKOPOLTFFTIALOSCHYORLPPHILWATGLKGSGSGSLPPLERL TL*

[0102] **MCP-***Jinke* **r-**hAPOBEC 1-NLS

MASNFTQFVLVDNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSSA QNRKYTIKVEVPKGAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPS AIAANSGIYGGSGSGAGSGSiM *GGGAPGSGGGSSGSETPGTSESATPESM*TS*EKG*PST **GDPTLRRRIEPWEFDVFYDPRELRKEACLLYEIKWGMSRKIWRSSGKNTTNHVEVNF** IKKFTSERDFFffSMSCSITWFLSWSPCWECSQAIREFLSRFffGVTLVIYVARLFWHMD **QQNRQGLRDLVNSGVTIQIMRASEYYHCWRNFVNYPPGDEAHWPQYPPLWMMLY** ALELHCIILSLPPCLKISRRWQNHLTFFRLFiLQNCHYQTIPPHILLATGLIFiPSVAWRGS GSGSPKKKRKV*

[0103] **MCP-***Jinke* **r-**hAPOBEC 1-NES

MASNFTQFVLVDNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSSA QNRKYTIKVEVPKGAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPS AIAANSGIYGGSGSGAGSGSiM *GGGAPGSGGGSSGSETPGTSESATPESM*TS*EK*GPST GDPTLRRRIEPWEFDVFYDPRELRKEACLLYEIKWGMSRKIWRSSGKNTTNHVEVNF IKKFTSERDFFffSMSCSITWFLSWSPCWECSQAIREFLSRFffGVTLVIYVARLFWHMD **QQNRQGLRDLVNSGVTIQIMRASEYYHCWRNFVNYPPGDEAHWPQYPPLWMMLY** ALELHCIILSLPPCLKISRRWQNHLTFFRLFiLQNCHYQTIPPHILLATGLIFiPSVAWRGS GSGSLPPLERLTL*

[0104] Alternate spacer (can be used in place of GGSGGSGGS):

SGSETPGTSESATPES

[0105] 3XNLS-4xlN-cdADAR2

MPKKKRKVDPKKKRKVDPKKKRKVGSYPYDVPDYAGSNARTRRRERRAEKQA QWKAANGGGGSGGGGSGGGGSNARTRRRERRAEKQAQWKAANGGGGSGGG GSGGGGSNARTRRRERRAEKQAQWKAANGGGGSGGGGSGGGGSNARTRRRE
RRAEKOAOWKAANLHLDQTPSRQPIPSEGLQLHLPQVLADAVSRLVLGKFGDLTD
NF SSPHARRKVLAGVVMTTGTDVKDAKVISVSTGTKCINGEYMSDRGLALNDCHAE
IISRRSLLRFLYTQLELYLNNKDDQKRSIFQKSERGGFRLKENVQFHLYISTSPCGDAR
IFSPHEPILEEPADRHPNRKARGQLRTKIESGEGTIPVRSNASIQTWDGVLQGERLLTM
SCSDKIARWNVVGIQGSLLSIFVEPIYFSSIILGSLYHGDHLSRAMYQRISNIEDLPPLY
TLNKPLLSGISNAEARQPGKAPNFSVNWTVGDSAIEVINATTGKDELGRASRLCKHA
LYCRWMRVHGKVPSHLLRSKITKPNVYHESKLAAKEYQAAKARLFTAFIKAGLGA
WVEKPTEQDQF SLTP

[0106] N22p-hAPOBECl

MPKKKRKVDGSGNARTRRRERRAEKQAQWKAANGGGGTSGSGSGSPAGGGA
PGSGGGSMTSEKGPSTGDPTLRRRIEPWEFDVFYDPRELRKEACLLYEIKWGMSRKI
WRSSGKNTTNHVEVNFIKKFTSERDFHPSMSCSITWFLSWSPCWECSQAIREFLSRHP
GVTLVIYVARLFWHMDOONROGLRDLVNSGVTIOIMRASEYYHCWRNFVNYPPGD
EAHWPQYPPLWMMLYALELHCIILSLPPCLKISRRWQNHLTFFRLHLQNCHYQTIPPH
ILLATGLIHPSVAWR

[0107] 3XNLS-4xlN-hAPOBECl

MPKKKRKVDPKKKRKVDPKKKRKVGSYPYDVPDYAGSNARTRRRERRAEKQA QWKAANGGGGSGGGGSGGGGSNARTRRRERRAEKQAQWKAANGGGGSGGG GSGGGGSNARTRRRERRAEKQAQWKAANGGGGSGGGGSGGGGSNARTRRRE
RRAEKOAOWKAANMTSEKGPSTGDPTLRRRIEPWEFDVFYDPRELRKEACLLYEIK
WGMSRKIWRSSGKNTTNHVEVNFIKKFTSERDFHPSMSCSITWFLSWSPCWECSQAI
REFLSRHPGVTLVIYVARLFWHMDQQNRQGLRDLVNSGVTIQIMRASEYYHCWRNF
WYPPGDEAHWPQYPPLWMMLYALELHCIILSLPPCLKISRRWQNHLTFFRLHLQNC
HYQTIPPHILLATGLIHPSVAWR

[0108] C-terminal ADAR2 (residues 1-138 deleted)

MLRSFVQFPNASEAHLAMGRTLSVNTDFTSDQADFPDTLFNGFETPDKAEPPFYVGS
NGDDSFSSSGDLSLSASPVPASLAQPPLPVLPPFPPPSGKNPVMILNELRPGLKYDFLS
ESGESHAKSFVMSVVVDGQFFEGSGRNKKLAKARAAQSALAAIFNLHLDQTPSRQPI
PSEGLQLHLPQVLADAVSRLVLGKFGDLTDNFSSPHARRKVLAGVVMTTGTDVKDA
KVISVSTGTKCrNGEYMSDRGLALNDCHAEIISRRSLLRFLYTQLELYLNNfCDDQKRS
IFQKSERGGFRLKENVQFHLYISTSPCGDARIFSPHEPILEEPADRHPNRKARGQLRTKI

ESGEGTIPVRSNASIQTWDGVLQGERLLTMSCSDKIARWNVVGIQGSLLSIFVEPIYFS SIILGSL YHGDHL SRAM YQRI SNIEDLPPL YTL NKPLL SGI SN AE ARQPGK AP NF SVNW TVGD SAIE VINATTGKDELGRASRLCKHAL YCRWMRVHGKVP SHLLRSKITKPNVY HESKLAAKEYQAAKARLFTAFIKAGLGAWVEKPTEQDQFSLTP*

[0109] MS2-RNA:

Single:

NNNNNNNNNNNNNNNNNNNggccAACATGAGGATCACCCATGTCTGCAGggcc

Dual:

aACATGAGGATCACCCATGTcNNNNNNNNNNNNNNNNNNNaACATGAGGATCA CCCATGTc

[0110] BoxB RNA:

Single:

NNNNNNNNNNNNNNNNNNNgggccctgaagaagggccc

Dual:

ggGCCCTGAAGAAGGGCccNNNNNNNNNNNNNNNNNNggGCCCTGAAGAAGG GCcc

[0111] PP7-RNA:

NNNNNNNNNNNNNNNNNNNccggagcagacgatatggcgtcgctccgg

[0112] Dual Hairpin RNA:

TGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACNNNNNNNNNN NNNNNNNNNNGTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCA C

[0113] A-U to G-C substitutions in adRNA

v 1 : GGGTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACCT

v 2 : GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCAC

v 3 : GTGGAASAGGAGAACAATATGCTAAATGTTGTTCTCGTCTCCCAC

[0114] dCas9Ci-NES-*Linker*-cdADAR2(E488Q)

MARILAFAIGISSIGWAFSENDELKDCGVRIFTKVENPKTGESLALPRRLARSAR
KRLARRKARLNHLKHLIANEFKLNYEDYQSFDESLAKAYKGSLISPYELRFRAL
NELLSKQDFARVILHIAKRRGYDDIKNSDDKEKGAILKAIKQNEEKLANYQSVG
EYLYKEYFQKFKENSKEFTNVRNKKESYERCIAQSFLKDELKLIFKKQREFGFSF
SKKFEEEVLSVAFYKRALKDFSHLVGNCSFFTDEKRAPKNSPLAFMFVALTRIIN
LLNNLKNTEGILYTKDDLNALLNEVLKNGTLTYKQTKKLLGLSDDYEFKGEKG
TYFIEFKKYKEFIKALGEHNLSQDDLNEIAKDITLIKDEIKLKKALAKYDLNQNQ
IDSLSKLEFKDHLNISFKALKLVTPLMLEGKKYDEACNELNLKVAINEDKKDFL
PAFNETYYKDEVTNPVVLRAIKEYRKVLNALLKKYGKVHKINIELAREVGKNHS
QRAKIEKEQNENYKAKKDAELECEKLGLKINSKNILKLRLFKEQKEFCAYSGEK
IKISDLQDEKMLEIDAIYPYSRSFDDSYMNKVLVFTKQNQEKLNQTPFEAFGNDS
AKWQKIEVLAKNLPTKKQKRILDKNYKDKEQKNFKDRNLNDTRYIARLVLNYT
KDYLDFLPLSDDENTKLNDTQKGSKVHVEAKSGMLTSALRHTWGFSAKDRNN
HLHHAIDAVIIAYANNSIVKAFSDFKKEQESNSAELYAKKISELDYKNKRKFFEPF
SGFRQKVLDKIDEIFVSKPERKKPSGALHEETFRKEEEFYQSYGGKEGVLKALE
LGKIRKVNGKIVKNGDMFRVDIFKHKKTNKFYAVPIYTMDFALKVLPNKAVAR
SKKGEIKDWILMDENYEFCFSLYKDSLILIQTKDMQEPEFVYYNAFTSSTVSLIVS
KHDNKFETLSKNQKILFKNANEKEVIAKSIGIQNLKVFEKYIVSALGEVTKAEFR
QREDFKKSGLPPLERLTLG^GGGGSQLHLPQVLADAVSRLVLGKFGDLTDNFSSPHA
RRKVLAGVVMTTGTDVKDAKVISVSTGTKCINGEYMSDRGLALNDCHAEIISRRSLL
RFLYTQLELYLNNKDDQKRSIFQKSERGGFRLKENVQFHLYISTSPCGDARIFSPHEPI
LEEPADRHPNRKARGQLRTKIESGQGTIPVRSNASIQTWDGVLQGERLLTMSCSDKIA
RWNVVGIQGSLLSIFVEPIYFSSIILGSLYHGDHLSRAMYQRISNIEDLPPLYTLNKPLL
SGISNAEARQPGKAPNFSVNWTVGDSAIEVINATTGKDELGRASRLCKHALYCRWM
RVHGKVPSHLLRSKITKPNVYHESKLAAKEYQAAKARLFTAFIKAGLGAWVEKPTE
QDQFSLT

[0115] Single and dual ADAR2 recruiting domain:

Single:

GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACACAAACC
GAGCGGTGTCTGT

Dual 1:
GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACCAAACCG
AGCGGTGTCTGTGGTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTAT
CCCAC

Dual 2:
GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACTACAAAC
CGAGCGGTGTCTGGTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTAT
CCCAC

Dual 3:
GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACTTTACAAA
CCGAGCGGTGTCGTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATC
CCAC

Dual 4:
GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACGTTTTACA
AACCGAGCGGTGGTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTAT
CCCAC

Dual 5:
GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACAAGTTTTA
CAAACCGAGCGGGTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTAT
CCCAC

BRIEF DESCRIPTION OF THE DRAWINGS

[0116] FIG. 1 is a schematic of the vector constructs developed for the delivery of the modified endogenous or orthogonal tRNA.

[0117] FIG. 2A-B show suppression efficiencies of the tRNA constructs: **(FIG. 2A)** Relative efficiencies of the suppressor tRNAs derived from arginine, serine and leucine towards the amber, ochre and opal stop codons; Representative images showing the restoration of GFP expression in the presence of the Ser tRNAAmber **(FIG. 2B)** Comparison of the suppression efficiencies of the single or dual pyrrolysyl tRNAs towards amber, ochre and opal stop codons in the presence of 2mM UAA; Representative images showing the

relative GFP restoration using single and dual pyrrolysyl tRNAAmber in the presence of 2mM UAA.

[0118] FIG. 3 shows the GFP reporter results for dystrophin with various tRNA and amino acids.

[0119] FIG. 4 shows the results of the dystrophin restoration experiments performed in mdx mice.

[0120] FIG. 5 shows sequences used to generate the ADAR2 constructs.

[0121] FIG. 6 shows non-limiting examples of RNA level point mutations to a codon that can be made by ADAR2.

[0122] FIG. 7 shows exemplary schematics of constructs that may be used in an ADAR2 based RNA editing system.

[0123] FIG. 8 shows the results of optimization of the length of adRNA and distance of the edit from the ADAR2 recruiting domain. The first number in the shorthand for each category on the Y-axis is the length of adRNA and the second number (following the dash) is the distance of edit from ADAR2 recruiting domain. 20-6 with ADAR2 recruiting region v2 gave us the best results.

[0124] FIG. 9 shows *in vitro* restoration of GFP expression using the editing systems described herein.

[0125] FIG. 10 shows the results of optimization of hairpins with mismatches. The first number in the shorthand for each category on the Y-axis is the number of mismatches and the second number is the number of bases it is from the target. For example, 13 is 1 mismatch, 3 bases away from the target.

[0126] FIG. 11 shows the results of varying lengths of toe hold, guide RNA sequences with no mismatches to the target.

[0127] **FIG. 12A-C** show results of **(FIG. 12A)** immunostaining, **(FIG. 12B)** Western blot, and **(FIG. 12C)** *in vitro* OTC mRNA editing assays.

[0128] **FIG. 13** is a Western blot that shows the restoration of dystrophin expression using suppressor tRNA, in comparison with the Cas9 based approaches.

[0129] **FIG. 14** shows normalized dystrophin mRNA levels.

[0130] **FIG. 15** shows results of immunostaining.

[0131] **FIG. 16A-D** shows *in vitro* suppression and editing of stop codons in GFP reporter mRNA: **(FIG. 16A)** Activity of arginine, serine and leucine suppressor tRNAs targeting amber, ochre and opal stop codons (n=3 independent replicates). **(FIG. 16B)** Orthogonal tRNA/aaRS (MbPylRS) based suppression of amber, ochre and opal stop codons in the presence of one or two copies of the pyrrolysyl-tRNA delivered via an AAV vector and in the presence of ImM Nε-Boc-L-Lysine (n=3 independent replicates) (p-values 0.022, 0.002, 0.027 respectively). **(FIG. 16C)** ADAR2 based RNA editing efficiencies of amber and ochre stop codons, in one-step, two-steps, or in combination with suppressor tRNAs (n=3 independent replicates). **(FIG. 16D)** ADAR2 based RNA editing efficiencies of amber and ochre stop codons in the presence of one or two copies of the adRNA, delivered via an AAV vector (n=3 or 6 independent replicates) (p-values 0.0003, 0.0001, 0.0015 respectively).

[0132] **FIG. 17A-E** shows *in vivo* RNA targeting in mouse models of human disease: **(FIG. 17A)** Schematic of the DNA and RNA targeting approaches to restore dystrophin expression in *mdx* mice: (i) a dual gRNA-CRISPR based approach leading to in frame excision of exon 23; (ii) tRNA suppression of the ochre codon; and (iii) ADAR2 based editing of the ochre codon. **(FIG. 17B)** Immunofluorescence staining for dystrophin and nNOS in controls and treated samples (scale bar: 250μm). **(FIG. 17C)** *In vivo* TAA->TGG/TAG/TGA RNA editing efficiencies in corresponding treated adult *mdx* mice (n=3 or 4 mice). **(FIG. 17D)** Schematic of the OTC locus in *spf* ^{sh} mice which have a G->A point mutation at a donor splice site or missense in the last nucleotide of exon 4, and approach for correction of mutant OTC mRNA via ADAR2 mediated RNA editing **(FIG. 17E)** *In vivo* A->G RNA editing efficiencies in corresponding treated adult *spf* ^{sh} mice (n=3 or 4 mice).

[0133] **FIG. 18A-B** show *in vitro* tRNA suppression evaluation and optimization: **(FIG. 18A)** Specificity of modified serine suppressor tRNAs for ochre and opal stop codons (n=3 independent replicates). **(FIG. 18B)** Ochre stop codon suppression efficiency utilizing three different aaRS: MbPylRS, MmPylRS and AcKRS, and two or four copies of the pyrroysyl-tRNA, or serine suppressor tRNA, all delivered using an AAV vector. MbPylRS, MmPylRS: ImM Ne-Boc-L-Lysine; AcKRS: 1 or lOmM Nε-Acetyl-L-Lysine (n=3 independent replicates).

[0134] **FIG. 19A-C** shows *in vitro* ADAR2 mediated site-specific RNA editing evaluation and optimization: **(FIG. 19A)** GFP expression is restored when adRNA/radRNA has two mismatches corresponding to the two adenosines in the ochre stop codon. Presence of a single mismatch results in the formation of an amber or opal stop codon (n=3 independent replicates). **(FIG. 19B)** Panel of adRNA designs used. **(FIG. 19C)** Optimization of adRNA antisense region using adRNA design 1: length and distance from the ADAR2 recruiting region were systematically varied, and editing efficiency calculated as a ratio of Sanger peak heights G/(A+G) (n=3 independent replicates).

[0135] **FIG. 20A-C** shows *in vivo* targeting of dystrophin mRNA via suppressor tRNAs: **(FIG. 20A)** Progressively increasing restoration of dystrophin expression over time in *mdx* mice treated with AAV8-dual-serine-ochre-tRNA. **(FIG. 20B)** UAA inducible nNOS localization in *mdx* mice treated with AAV8-dual-pyrrolysine-ochre-tRNA-MbPylRS. **(FIG. 20C)** Western blot for dystrophin shows partial recovery of dystrophin expression in the *mdx* mice treated with a serine tRNA ochre, the pyrrolysyl-tRNA ochre and administered with the UAA, as well as in Cas9/gRNAs treated samples.

[0136] **FIG. 21A-D** show *in vitro* and *in vivo* editing of dystrophin and OTC mRNA: **(FIG. 21A)** Representative Sanger sequencing plot showing 12.7% editing of the ochre stop codon (TAA->TGG) in a fragment of the *mdx* dystrophin mRNA expressed in HEK 293T cells (quantified using NGS). **(FIG. 21B)** Representative example of *in vivo* RNA editing analyses of treated *mdx* mouse (quantified using NGS). **(FIG. 21C)** Representative Sanger sequencing plot showing 29.7% correction of the point mutation in a fragment of the *spf* ^{sh} OTC mRNA

expressed in HEK 293T cells (quantified using NGS). **(FIG. 21D)** Representative example of *in vivo* RNA editing analyses of treated *spf* ^{sh} mouse (quantified using NGS).

[0137] **FIG. 22A-B** show *in vitro* editing efficiency of ADAR2-E488Q. ADAR2-E488Q enables higher efficiency than the ADAR2 in the *in vitro* editing of: **(FIG. 22A)** a fragment of *spf* ^{sh} OTC mRNA expressed in HEK293T cells (n=3 independent replicates) (p-value 0.037), and **(FIG. 22B)** a fragment of *mdx* dystrophin mRNA expressed in HEK293T cells (n=3 independent replicates) (p-values 0.048, 0.012 respectively). Efficiency was calculated as a ratio of Sanger peak heights G/(A+G).

[0138] **FIG. 23A-D** show schematics of **(FIG. 23A)** MCP or N22 fusions with ADARl or ADAR2, **(FIG. 23B)** recruitment of APOBEC by adRNA, **(FIG. 23C)** a more general adRNA architecture, and **(FIG. 23D)** the structure of the v2 adRNA scaffold after folding.

[0139] **FIG. 24A-B** show schematics of optional embodiments in which **(FIG. 24A)** endogenous ADAR2 can be used in the methods disclosed herein in tissues with high endogenous ADAR2, *e.g.*, brain, lung, and spleen and **(FIG. 24B)** ADARl and/or ADAR2 levels can be increased in tissues with low levels of endogenous ADARl and ADAR2. Clockwise from the left, (1) delivery of adRNA and ADAR2 would result in high levels of RNA editing, (2) delivery of adRNA alone is likely to bring about little or no editing due to the low levels of endogenous ADARl and ADAR2, (3) treatment of cells with IFNs will lead to an increase in the ADARl (pi 50) levels but is unlikely to bring about any editing of the RNA target in the absence of the adRNA; (4) treatment of cells with IFNs with the addition of adRNA will lead to elevated levels of ADARl (pi 50) and in the presence of adRNA, is likely to lead to high levels of target RNA editing, (5) treatment of cells with IFNs with the addition of adRNA and ADAR2 will lead to elevated levels of ADARl expression, and high levels of RNA editing.

[0140] **FIG. 25** shows the rate of UAA to UAG conversion. The UAA is converted to UAG via ADAR2 based editing and addition of suppressor tRNA targeting the UAG stop codon led to partial restoration of GFP expression

[0141] **FIG. 26** shows the results of *in vivo* RNA editing in the mdx mouse model of muscular dystrophy.

[0142] **FIG. 27** shows the resulting edited sequences resulting from use of the promiscuous C-terminal ADAR2.

[0143] **FIG. 28** shows editing efficiency of the stabilized scaffolds.

[0144] **FIG. 29** shows the fraction of edited mRNA with single versus dual ADAR2 recruiting domains and the corresponding sequences.

[0145] **FIG. 30** shows the fraction of edited mRNA with various MCP-ADAR scaffolds.

[0146] **FIG. 31** shows alternative splice variants of OTC and is taken from Hodges, P.E. & Rosenberg, L.E. The spfash mouse: a missense mutation in the ornithine transcarbamylase gene also causes aberrant mRNA splicing. *Proc. Natl. Acad. Sci. U.S.A.* **86,** 4142-4146 (1989).

DETAILED DESCRIPTION

[0147] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. All nucleotide sequences provided herein are presented in the 5' to 3' direction. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All technical and patent publications cited herein are incorporated herein by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0148] The practice of the present technology will employ, unless otherwise indicated, conventional techniques of tissue culture, immunology, molecular biology, microbiology, cell biology, and recombinant DNA, which are within the skill of the art. *See, e.g.,* Sambrook and Russell eds. (2001) *Molecular Cloning: A Laboratory Manual,* 3rd edition; the series

Ausubel *etal.* eds. (2007) *Current Protocols in Molecular Biology;* the series *Methods in Enzymology* (Academic Press, Inc., N.Y.); MacPherson *et al.* (1991) *PCR 1: A Practical Approach* (IRL Press at Oxford University Press); MacPherson *et al.* (1995) *PCR 2: A Practical Approach;* Harlow and Lane eds. (1999) *Antibodies, A Laboratory Manual;* Freshney (2005) *Culture of Animal Cells: A Manual of Basic Technique,* 5th edition; Gait ed. (1984) *Oligonucleotide Synthesis;* U.S. Patent No. 4,683,195; Hames and Higgins eds. (1984) *Nucleic Acid Hybridization;* Anderson (1999) *Nucleic Acid Hybridization;* Hames and Higgins eds. (1984) *Transcription and Translation; Immobilized Cells and Enzymes* (IRL Press (1986)); Perbal (1984) *A Practical Guide to Molecular Cloning;* Miller and Calos eds. (1987) *Gene Transfer Vectorsfor Mammalian Cells* (Cold Spring Harbor Laboratory); Makrides ed. (2003) *Gene Transfer and Expression in Mammalian Cells;* Mayer and Walker eds. (1987) *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); and Herzenberg *et al.* eds (1996) *Weir's Handbook of Experimental Immunology.*

[0149] The terminology used in the description herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety.

[0150] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 1.0 or 0.1, as appropriate or alternatively by a variation of +/- 15 %, or alternatively 10% or alternatively 5% or alternatively 2%. It is to be understood, although not always explicitly stated, that all numerical designations are preceded by the term "about". It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[0151] Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the disclosure also contemplates that in some embodiments, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically intended that any of A, B

or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

[0152] Unless explicitly indicated otherwise, all specified embodiments, features, and terms intend to include both the recited embodiment, feature, or term and biological equivalents thereof.

[0153] ***Definitions***

[0154] As used in the specification and claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a polypeptide" includes a plurality of polypeptides, including mixtures thereof.

[0155] The term "about," as used herein when referring to a measurable value such as an amount or concentration and the like, is meant to encompass variations of 20%, 10%, 5%, 1 %, 0.5%, or even 0.1 % of the specified amount.

[0156] As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but do not exclude others. "Consisting essentially of when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination for the intended use. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. "Consisting of shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

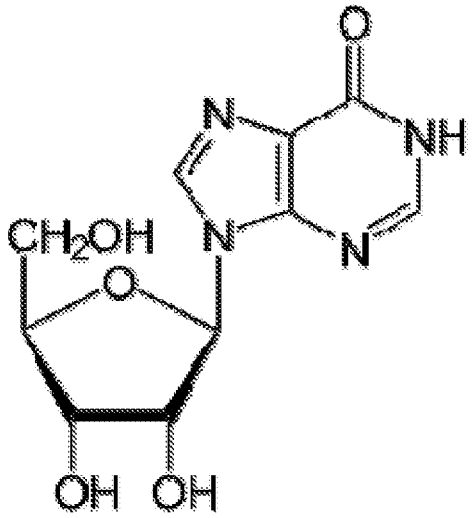
[0157] A "subject" of diagnosis or treatment is a cell or an animal such as a mammal, or a human. Non-human animals subject to diagnosis or treatment and are those subject to infections or animal models, for example, simians, murines, such as, rats, mice, chinchilla, canine, such as dogs, leporids, such as rabbits, livestock, sport animals, and pets.

[0158] The term "protein", "peptide" and "polypeptide" are used interchangeably and in their broadest sense to refer to a compound of two or more subunit amino acids, amino acid

analogs or peptidomimetics. The subunits may be linked by peptide bonds. In another embodiment, the subunit may be linked by other bonds, e.g., ester, ether, etc. A protein or peptide must contain at least two amino acids and no limitation is placed on the maximum number of amino acids which may comprise a protein's or peptide's sequence. As used herein the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D and L optical isomers, amino acid analogs and peptidomimetics. As used herein, the term "fusion protein" refers to a protein comprised of domains from more than one naturally occurring or recombinantly produced protein, where generally each domain serves a different function. In this regard, the term "linker" refers to a protein fragment that is used to link these domains together - optionally to preserve the conformation of the fused protein domains and/or prevent unfavorable interactions between the fused protein domains which may compromise their respective functions.

[0159] The terms "polynucleotide" and "oligonucleotide" are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides or analogs thereof. Polynucleotides can have any three-dimensional structure and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, RNAi, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure can be imparted before or after assembly of the polynucleotide. The sequence of nucleotides can be interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of this invention that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

[0160] A polynucleotide is composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); thymine (T); and uracil (U) for thymine when the polynucleotide is RNA. In some embodiments, the polynucleotide may comprise one or more other nucleotide bases, such as inosine (I), a nucleoside formed when hypoxanthine is attached to ribofuranose via a P-N9-glycosidic bond, resulting in the chemical structure:



Inosine is read by the translation machinery as guanine (G). The term "polynucleotide sequence" is the alphabetical representation of a polynucleotide molecule. This alphabetical representation can be input into databases in a computer having a central processing unit and used for bioinformatics applications such as functional genomics and homology searching.

[0161] As used herein, "expression" refers to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in an eukaryotic cell.

[0162] The terms "equivalent" or "biological equivalent" are used interchangeably when referring to a particular molecule, biological, or cellular material and intend those having minimal homology while still maintaining desired structure or functionality.

[0163] The term "encode" as it is applied to polynucleotides refers to a polynucleotide which is said to "encode" a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the

mRNA for the polypeptide and/or a fragment thereof. The antisense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

[0164] As used herein, the term "functional" may be used to modify any molecule, biological, or cellular material to intend that it accomplishes a particular, specified effect.

[0165] As used herein, the terms "treating," "treatment" and the like are used herein to mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease, disorder, or condition or sign or symptom thereof, and/or may be therapeutic in terms of a partial or complete cure for a disorder and/or adverse effect attributable to the disorder.

[0166] "Administration" can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. Suitable dosage formulations and methods of administering the agents are known in the art. Route of administration can also be determined and method of determining the most effective route of administration are known to those of skill in the art and will vary with the composition used for treatment, the purpose of the treatment, the health condition or disease stage of the subject being treated, and target cell or tissue. Non-limiting examples of route of administration include oral administration, nasal administration, injection, and topical application.

[0167] The term "effective amount" refers to a quantity sufficient to achieve a desired effect. In the context of therapeutic or prophylactic applications, the effective amount will depend on the type and severity of the condition at issue and the characteristics of the individual subject, such as general health, age, sex, body weight, and tolerance to pharmaceutical compositions. In the context of an immunogenic composition, in some embodiments the effective amount is the amount sufficient to result in a protective response against a pathogen. In other embodiments, the effective amount of an immunogenic composition is the amount sufficient to result in antibody generation against the antigen. In

some embodiments, the effective amount is the amount required to confer passive immunity on a subject in need thereof. With respect to immunogenic compositions, in some embodiments the effective amount will depend on the intended use, the degree of immunogenicity of a particular antigenic compound, and the health/responsiveness of the subject's immune system, in addition to the factors described above. The skilled artisan will be able to determine appropriate amounts depending on these and other factors.

[0168] In the case of an in vitro application, in some embodiments the effective amount will depend on the size and nature of the application in question. It will also depend on the nature and sensitivity of the in vitro target and the methods in use. The skilled artisan will be able to determine the effective amount based on these and other considerations. The effective amount may comprise one or more administrations of a composition depending on the embodiment.

[0169] The term "Cas9" refers to a CRISPR associated endonuclease referred to by this name (for example, UniProtKB G3ECR1 (CAS9_STRTR)) as well as dead Cas9 or dCas9, which lacks endonuclease activity *(e.g.,* with mutations in both the RuvC and HNH domain). The term "Cas9" may further refer to equivalents of the referenced Cas9 having at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity thereto, including but not limited to other large Cas9 proteins. In some embodiments, the Cas9 is derived from *Campylobacterjejuni* or another Cas9 otrholog 1000 amino acids or less in length.

[0170] The term "vector" refers to a polynucleotide (usually DNA) used to artificially carry foreign genetic material to another cell where it can be replicated or expressed. Non-limiting exemplary vectors include plasmids, viral vectors, cosmids, and artificial chromosomes. Such vectors may be derived from a variety of sources, including bacterial and viral sources. A non-limiting exemplary viral source for a plasmid is adeno-associated virus.

[0171] As used herein, the term "recombinant expression system" refers to a genetic construct or constructs for the expression of certain genetic material formed by recombination; the term "construct" in this regard is interchangeable with the term "vector" as defined herein.

[0172] The term "adeno-assocaited virus" or "AAV" as used herein refers to a member of the class of viruses associated with this name and belonging to the genus dependoparvovirus, family Parvoviridae. Multiple serotypes of this virus are known to be suitable for gene delivery; all known serotypes can infect cells from various tissue types. At least 11, sequentially numbered, are disclosed in the prior art. Non-limiting exemplary serotypes useful for the puposes disclosed herein include any of the 11 serotypes, e.g., AAV2 and AAV8.

[0173] The term "lentivirus" as used herein refers to a member of the class of viruses associated with this name and belonging to the genus lentivirus, family Retroviridae. While some lentiviruses are known to cause diseases, other lentivirus are known to be suitable for gene delivery. *See, e.g.,* Tomas et al. (2013) Biochemistry, Genetics and Molecular Biology: "Gene Therapy - Tools and Potential Applications," ISBN 978-953-51-1014-9, DOI: 10.5772/52534.

[0174] As used herein the term "restoring" in relation to expression of a protein refers to the ability to establish expression of full length protein where previously protein expression was truncated due to mutation.

[0175] The term "mutation" as used herein, refers to an alteration to a nucleic acid sequence encoding a protein relative to the consensus sequence of said protein. "Missense" mutations result in the substitution of one codon for another; "nonsense" mutations change a codon from one encoding a particular amino acid to a stop codon. Nonsense mutations often result in truncated translation of proteins. "Silent" mutations are those which have no effect on the resulting protein. As used herein the term "point mutation" refers to a mutation affecting only one nucleotide in a gene sequence. "Splice site mutations" are those mutations present pre-mRNA (prior to processing to remove introns) resulting in mistranslation and often truncation of proteins from incorrect delineation of the splice site.

[0176] "Messenger RNA" or "mRNA" is a nucleic acid molecule that is transcribed from DNA and then processed to remove non-coding sections known as introns. The resulting mRNA is exported from the nucleus (or another locus where the DNA is present) and

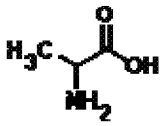
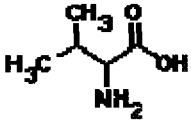
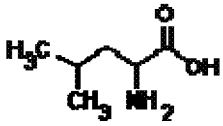
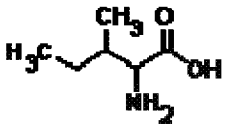
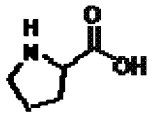
translated into a protein. The term "pre-mRNA" refers to the strand prior to processing to remove non-coding sections.

[0177] "Transfer ribonucleic acid" or "tRNA" is a nucleic acid molecule that helps translate mRNA to protein. tRNA have a distinctive folded structure, comprising three hairpin loops; one of these loops comprises a "stem" portion that encodes an anticodon. The anticodon recognizes the corresponding codon on the mRNA. Each tRNA is "charged with" an amino acid corresponding to the mRNA codon; this "charging" is accomplished by the enzyme tRNA synthetase. Upon tRNA recognition of the codon corresponding to its anticodon, the tRNA transfers the amino acid with which it is charged to the growing amino acid chain to form a polypeptide or protein. Endogenous tRNA can be charged by endogenous tRNA synthetase. Accordingly, endogenous tRNA are typically charged with canonical amino acids. Orthogonal tRNA, derived from an external source, require a corresponding orthogonal tRNA synthetase. Such orthogonal tRNAs may be charged with both canonical and non-canonical amino acids. In some embodiments, the amino acid with which the tRNA is charged may be detectably labeled to enable detection *in vivo*. Techniques for labeling are known in the art and include, but are not limited to, click chemistry wherein an azide/alkyne containing unnatural amino acid is added by the orthogonal tRNA/synthetase pair and, thus, can be detected using alkyne/azide comprising fluorophore or other such molecule.

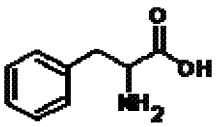
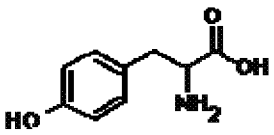
[0178] The term "stop codon" intends a three nucleotide contiguous sequence within messenger RNA that signals a termination of translation. Non-limiting examples include in RNA, UAG, UAA, UGA and in DNA TAG, TAA or TGA. Unless otherwise noted, the term also includes nonsense mutations within DNA or RNA that introduce a premature stop codon, causing any resulting protein to be abnormally shortened. tRNA that correspond to the various stop codons are known by specific names: amber (UAG), ochre (UAA), and opal (UGA).

[0179] "Canonical amino acids" refer to those 20 amino acids found naturally in the human body shown in the table below with each of their three letter abbreviations, one letter abbreviations, structures, and corresponding codons:

non-polar, aliphatic residues

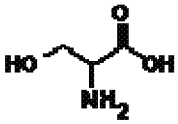
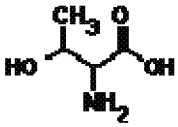
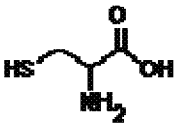
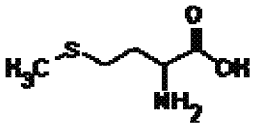
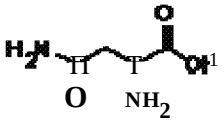
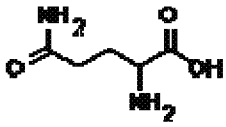
Glycine	Gly	G		GGU GGC GGA GGG
Alanine	Ala	A		GCU GCC GCA GCG
Valine	Val	V		GUU GUC GUA GUG
Leucine	Leu	L		UUA UUG CUU CUC CUA CUG
Isoleucine	Ile	I		AUU AUC AUA
Proline	Pro	P		CCU CCC CCA CCG

aromatic residues

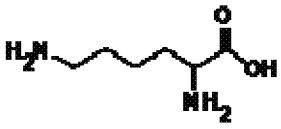
Phenylalanine	Phe	F		UUU UUC
Tyrosine	Tyr	Y		UAU UAC

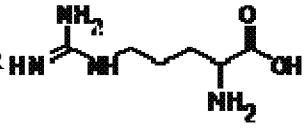
Tryptophan	Trp	W		UGG

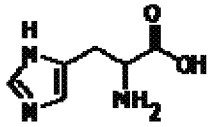
polar, non-charged residues

Serine	Ser	S		UCU UCC UCA UCG AGU AGC
Threonine	Thr	T		ACU ACC ACA ACG
Cysteine	Cys	C		UGU UGC
Methionine	Met	M		AUG
Asparagine	Asn	**N**		AAU AAC
Glutamine	Gin	Q		CAA CAG

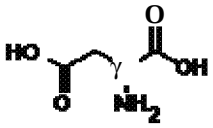
positively charged residues

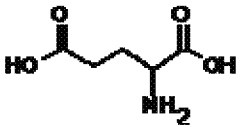
Lysine Lys K  AAA **AAG**

Arginine Arg **R**  CGU CGC CGA CGG AGA AGG

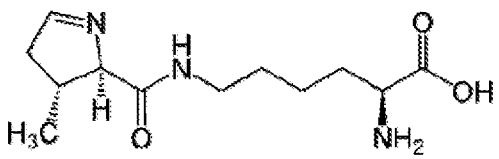
Histidine His H  CAU CAC

negatively charged residues

Aspartate Asp D  GAU GAC

Glutamate Glu E  GAA GAG

[0180] The term "non-canonical amino acids" refers to those synthetic or otherwise modified amino acids that fall outside this group, typically generated by chemical synthesis or modification of canonical amino acids ***(e.g.*** amino acid analogs). The present disclosure employs proteinogenic non-canonical amino acids in some of the methods and vectors disclosed herein. A non-limiting exemplary non-canonical amino acid is pyrrolysine (Pyl or O), the chemical structure of which is provided below:



Inosine (I) is another exemplary non-canonical amino acid, which is commonly found in tRNA and is essential for proper translation according to "wobble base pairing." The structure of inosine is provided above.

[0181] The term "ADAR" as used herein refers to an adenosine deaminase that can convert adenosines (A) to inosines (I) in an RNA sequence. ADAR1 and ADAR2 are two exemplary species of ADAR that are involved in mRNA editing *in vivo*. Non-limiting exemplary sequences for ADARl may be found under the following reference numbers: HGNC: 225; Entrez Gene: 103; Ensembl: ENSG 00000160710; OMTM: 146920; UniProtKB: P55265; and GeneCards: GC01M154554, as well as biological equivalents thereof. Non-limiting exemplary sequences for ADAR2 may be found under the following reference numbers: HGNC: 226; Entrez Gene: 104; Ensembl: ENSG00000197381; OMTM: 601218; UniProtKB: P78563; and GeneCards: GC21P045073, as well as biological equivalents thereof. Further non-limitined exemplary sequences of the catalytic domain are provided hereinabove. The forward and reverse RNA used to direct site-specific ADAR editing are known as "adRNA" and "radRNA," respectively. The catalytic domains of ADARl and ADAR2 are comprised in the sequences provided herein below.

[0182] ADARl catayltic domain:

KAERMGFTEVTPVTGASLRRTMLLLSRSPEAQPKTLPLTGSTFHDQIAMLSHRCFNTL
TNSFQP SLLGRKIL AAIFMKKD SEDMGVVVSLGTGNRC VKGD SL SLKGET VNDCHAE
IISRRGFIRFLYSELMKYNSQTAKDSIFEPAKGGEKLQIKKTVSFHLYISTAPCGDGALF
DK SC SDRAME STE SRHYP VFENPKQGKLRTK VENGEGTIP VES SDIVPT WDGIRLGER
LRTMSCSDKILRWNVLGLQGALLTHFLQPIYLKSVTLGYLFSQGHLTRAICCRVTRD
GSAFEDGLRHPFIVNHPKVGRVSIYDSKRQSGKTKETSVNWCLADGYDLEILDGTRG
TVDGPRNEL SRVSKKNIFLLFKKLC SFRYRT<DLLRLSYGEAKK AARD YETAKNYFKK
GLKDMGYGNWISKPQEEKNFYLCPV

[0183] ADAR2 catalytic domain:

QLHLPQVLADAVSRLVLGKFGDLTDNFSSPHARRKVLAGVVMTTGTDVKDAKVISV
STGTKCINGEYMSDRGLALNDCHAEIISRRSLLRFLYTQLELYLNNKDDQKRSIFQKS
ERGGFRLKENVQFHLYISTSPCGDARIFSPHEPILEEPADRHPNRKARGQLRTKIESGE
GTIPVRSNASIQTWDGVLQGERLLTMSCSDKIARWNVVGIQGSLLSIFVEPIYFSSIILG
SL YHGDHL SRAM YQRI SNIEDLPPL YTLNKPLL SGI SN AE ARQPGK APNF SVNWT VG
DSAIEVINATTGKDELGRASRLCKHALYCRWMRVHGKVPSHLLRSKITKPNVYHESK
LAAKEYQAAKARLFTAFIKAGLGAWVEKPTEQDQFSLT

[0184] The double stranded RNA binding domains (dsRBD) of an ADAR is comprised in the sequence provided herein below.

[0185] ADAR dsRBD:

MDIEDEENMS SSSTDVKENRNLDNVSPKDGSTPGPGEGSQLSNGGGGGPGRKRPLEE
GSNGHSKYRLKKRRKTPGPVLPKNALMQLNEIKPGLQYTLLSQTGPVHAPLFVMSV
EVNGQWEGSGPTKKKAKLHAAEKALRSFVQFPNASEAHLAMGRTLSVNTDFTSDQ
ADFPDTLFNGFETPDKAEPPFYVGSNGDDSFSSSGDLSLSASPVPASLAQPPLPVLPPF
PPPSGKNPVMILNELRPGLKYDFLSESGESHAKSFVMSVVVDGQFFEGSGRNKKLAK
ARAAQ SAL AAIFN

[0186] It is appreciated that further mutations can be made to the sequence of the ADAR and/or its various domains. For example, Applicants have generated E488Q and E1008Q mutants of both ADARl and ADAR2, as well as a "promiscuous" variant of ADAR2 - resulting from a C-terminal deletion. This "promiscuous" variant is known as such because it demonstrated promiscuity in edited reads with several As close to a target sequence showing an A to G conversion (verified across 2 different loci). The sequence of this variant is provided herein below.

[0187] "Promiscuous" ADAR2 variant:

MLRSFVQFPNASEAHLAMGRTLSVNTDFTSDQADFPDTLFNGFETPDKAEPPFYVGS
NGDDSFSSSGDLSLSASPVPASLAQPPLPVLPPFPPPSGKNPVMILNELRPGLKYDFLS
ESGESHAKSFVMSVVVDGQFFEGSGRNKKLAKARAAQSALAAIFNLHLDQTPSRQPI
PSEGLQLHLPQVLADAVSRLVLGKFGDLTDNFSSPHARRKVLAGVVMTTGTDVKDA
KVISVSTGTKCINGEYMSDRGLALNDCHAEIISRRSLLRFLYTQLELYLNNKDDQKRS
IFQKSERGGFRLKENVQFHLYISTSPCGDARIFSPHEPILEEPADRHPNRKARGQLRTKI
ESGEGTIPVRSNASIQTWDGVLQGERLLTMSCSDKIARWNVVGIQGSLLSIFVEPIYFS
SIILGSLYHGDHLSRAMYQRISNIEDLPPLYTLNKPLLSGISNAEARQPGKAPNFSVNW
TVGDSAIEVINATTGKDELGRASRLCKHAL YCRWMRVHGKVP SHLLRSKITKPNVY
HESKLAAKEYQAAKARLFTAFIKAGLGAWVEKPTEQDQFSLTP*

Not to be bound by theory, a C-terminal deletion in ADARl may produce the same or similar effect.

[0188] The term "deficiency" as used herein refers to lower than normal (physiologically acceptable) levels of a particular agent. In context of a protein, a deficiency refers to lower than normal levels of the full length protein.

[0189] The term "dystrophin" as used herein refers to the protein corresponding with that name and encoded by the gene *Dmd*; a non-limiting example of which is found under UniProt Reference Number P I 1532 (for humans) and P I 153 1 (for mice).

[0190] The term "ornithine transcarbamylase" or "OTC" as used herein refers to the protein corresponding with that name and encoded by the gene *Otc*; a non-limiting example of which is found under UniProt Reference Number P00480 (for humans) and P I 1725 (for mice). OTC deficiency is an X-linked genetic condition resulting in high concentrations of ammonia in blood. In some cases, OTC deficiency is caused by a G->A splice site mutation in the donor splice site of exon 4 that results in mis-splicing of the pre-mRNA. This mutation results in the formation of a protein that either is elongated or bears a point mutation. There is a 15-20 fold reduction in the OTC protein levels. The **FIG. 31** (taken from Hodges, P. E. & Rosenberg, L. E. The spfash mouse: a missense mutation in the ornithine transcarbamylase gene also causes aberrant mRNA splicing. *Proc. Natl. Acad. Sci. U. S. A.* **86,** 4142-4146 (1989)) shows the alternative forms produced. The sequences thereof are provided below:

OTC pre -mRNA (wild type) :
. . . . . CTCACAGACACCGCTCGGTTTGTAAAACTTTTCTTC. . . . .
OTC pre -mRNA (mutant) :
. . . . . ***CTCACAGACACCGCTCAGTTTGTAAAACTTTTCTTC*** . . . .
OTC mRNA (incorrectly spliced, mutant) :
. . . . . CTCACAGACACCGCTCAGTTTGTAAAACTTTTCTTC. . . . .
OTC mRNA (correctly spliced, mutant) :
. . . . . CTCACAGACACCGCJYJATGTCTJATCTAGCATGACA. . . .
OTC mRNA (correctly spliced, wild type)
. . . . . CTCACAGACACCGCTCGTGTCTTATCTAGCATGACA . . . .

As shown above, a correct splice variant may be produced when the mutation is present; however, such production results in a missense mutation, which also can contribute to OTC deficiency.

[0191] The terms "hairpin," "hairpin loop," "stem loop," and/or "loop" used alone or in combination with "motif is used in context of an oligonucleotide to refer to a structure formed in single stranded oligonucleotide when sequences within the single strand which are

complementary when read in opposite directions base pair to form a region whose conformation resembles a hairpin or loop.

[0192] As used herein, the term "domain" refers to a particular region of a protein or polypeptide and is associated with a particular function. For example, "a domain which associates with an RNA hairpin motif refers to the domain of a protein that binds one or more RNA hairpin. This binding may optionally be specific to a particular hairpin. For example, the M2 bacteriophage coat protein (MCP) is capable of specifically binding to particular stem-loop structures, including but not limited to the MS2 stem loop. *See, e.g.* Peabody, D.S., "The RNA binding site of bacteriophage MS2 coat protein." *EMBO J*. 12(2):595-600 (1993); Corrigan and Chubb, "Biophysical Methods in Cell Biology" *Methods in Cell Biology* (2015). Similarly, λ N22 - referred to herein as "N22 peptide" is capable of specifically binding to particular stem-loop structures, including but not limited to BoxB stem loops. *See, e.g.,* Cilley and Williamson, "Analysis of bacteriophage N protein and peptide binding to boxB RNA using polyacrylamide gell coelectrophoresis (PACE)." *RNA* 3(l):57-67 (1997). The sequences of both MCP and MS2 stem loop and N22 peptide and BoxB loop are provided hereinabove in context of fusion proteins with an ADAR (MCP, N22 peptide) and use in adRNA (MS2 stem loop, BoxB loop), respectively.

[0193] The term "APOBEC" as used herein refers to any protein that falls within the family of evolutionarily conserved cytidine deaminases involved in mRNA editing - catalyzing a C to U conversion - and equivalents thereof. In some aspects, the term APOBEC refers to any one of APOBEC1, APOBEC2, APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3E, APOBEC3F, APOBEC3G, APOBEC3H, APOBEC4, or equivalents each thereof. Non-limiting exemplary sequences of fusion proteins comprising one or more APOBEC domains are provided herein both fused to an ADAR domain or fused to alternative domains to render them suitable for use in an RNA editing system. To this end, APOBECs can be considered an equivalent of ADAR - catalyzing editing albeit by a different conversion. Thus, not to be bound by theory, Applicants believe that all embodiments contemplated herein for use with an ADAR based editing system may be adapted for use in an APOBEC based RNA editing system.

[0194] As used herein, the term "interferon" refers to a group of signaling proteins known to be associated with the immune response. In context of this application, the interferons of interest are those that result in enhanced expression of an ADAR. The correlation between interferon a and ADARl is well known, and, thus, the present disclosure contemplates use of interferon a as a means of increasing endogenous ADARl expression. Commercial sources of isolated or recombinant interferon a include but are not limited to Sigma-Aldrich, R&D Sytems, Abeam, and Thermo Fisher Scientific. Alternatively, interferon a may be produced using a known vector and given protein sequence, *e.g.*Q6QNB6 (human IFNA).

[0195] It is to be inferred without explicit recitation and unless otherwise intended, that when the present disclosure relates to a polypeptide, protein, polynucleotide or antibody, an equivalent or a biologically equivalent of such is intended within the scope of this disclosure. As used herein, the term "biological equivalent thereof is intended to be synonymous with "equivalent thereof when referring to a reference protein, antibody, polypeptide or nucleic acid, intends those having minimal homology while still maintaining desired structure or functionality. Unless specifically recited herein, it is contemplated that any polynucleotide, polypeptide or protein mentioned herein also includes equivalents thereof. For example, an equivalent intends at least about 70% homology or identity, or at least 80 % homology or identity and alternatively, or at least about 85 %, or alternatively at least about 90 %, or alternatively at least about 95 %, or alternatively 98 % percent homology or identity and exhibits substantially equivalent biological activity to the reference protein, polypeptide or nucleic acid. Alternatively, when referring to polynucleotides, an equivalent thereof is a polynucleotide that hybridizes under stringent conditions to the reference polynucleotide or its complement.

[0196] Applicants have provided herein the polypeptide and/or polynucleotide sequences for use in gene and protein transfer and expression techniques described below. It should be understood, although not always explicitly stated that the sequences provided herein can be used to provide the expression product as well as substantially identical sequences that produce a protein that has the same biological properties. These "biologically equivalent" or "biologically active" polypeptides are encoded by equivalent polynucleotides as described herein. They may possess at least 60%, or alternatively, at least 65%, or alternatively, at least

70%, or alternatively, at least 75%, or alternatively, at least 80%, or alternatively at least 85%, or alternatively at least 90%, or alternatively at least 95% or alternatively at least 98%, identical primary amino acid sequence to the reference polypeptide when compared using sequence identity methods run under default conditions. Specific polypeptide sequences are provided as examples of particular embodiments. Modifications to the sequences to amino acids with alternate amino acids that have similar charge. Additionally, an equivalent polynucleotide is one that hybridizes under stringent conditions to the reference polynucleotide or its complement or in reference to a polypeptide, a polypeptide encoded by a polynucleotide that hybridizes to the reference encoding polynucleotide under stringent conditions or its complementary strand. Alternatively, an equivalent polypeptide or protein is one that is expressed from an equivalent polynucleotide.

[0197] "Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogstein binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PC reaction, or the enzymatic cleavage of a polynucleotide by a ribozyme.

[0198] Examples of stringent hybridization conditions include: incubation temperatures of about 25°C to about 37°C; hybridization buffer concentrations of about 6x SSC to about lOx SSC; formamide concentrations of about 0% to about 25%; and wash solutions from about 4x SSC to about 8x SSC. Examples of moderate hybridization conditions include: incubation temperatures of about 40°C to about 50°C; buffer concentrations of about 9x SSC to about 2x SSC; formamide concentrations of about 30% to about 50%; and wash solutions of about 5x SSC to about 2x SSC. Examples of high stringency conditions include: incubation temperatures of about 55°C to about 68°C; buffer concentrations of about lx SSC to about O.lx SSC; formamide concentrations of about 55% to about 75%; and wash solutions of about lx SSC, O.lx SSC, or deionized water. In general, hybridization incubation times are from 5 minutes to 24 hours, with 1, 2, or more washing steps, and wash incubation times are

about 1, 2, or 15 minutes. SSC is 0.15 M NaCl and 15 mM citrate buffer. It is understood that equivalents of SSC using other buffer systems can be employed.

[0199] "Homology" or "identity" or "similarity" refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An "unrelated" or "non-homologous" sequence shares less than 40% identity, or alternatively less than 25% identity, with one of the sequences of the present invention.

Modes of Carrying Out the Disclosure

[0200] Point mutations underlie many genetic diseases. In this regard, while programmable DNA nucleases have been used to repair mutations, their use for gene therapy poses multiple challenges: one, efficiency of homologous recombination is typically low in cells; two, an active nuclease presents a risk of introducing permanent off-target mutations; and three, prevalent programmable nucleases typically comprise elements of non-human origin raising the potential of *in vivo* immunogenicity. In light of these, approaches to instead directly target RNA, and use of molecular machinery native to the host would be highly desirable. Towards this, Applicants have engineered and optimized two complementary approaches, referred together hereon as tRiAD, based on the use of tRNAs in codon suppression and adenosine deaminases in RNA editing. Specifically, by delivering modified endogenous tRNAs or the RNA editing enzyme ADAR and an associated guiding RNA (adRNA) via adeno-associated viruses, Applicants enabled premature stop codon read-through and correction in the *mdx* mouse model of muscular dystrophy that harbors a nonsense mutation in the dystrophin gene. Additionally, Applicants engineered ADAR2 mediated correction of a point mutation in liver RNA of the *spf* ^{sh} mouse model of ornithine transcarbamylase (OTC) deficiency. Taken together, the results disclosed herein establish the use of suppressor tRNAs and ADAR2 for *in vivo* RNA targeting, and this integrated tRiAD approach is robust, genomically scarless, and potentially non-immunogenic, as it utilizes effector RNAs and human proteins.

[0201] Aspects of the disclosure relate to a tRNA based protein editing system optionally alone or in combination with an ADAR based RNA editing system comprising one or more forward guide RNAs for the ADAR ("adRNAs") and one or more corresponding reverse guide RNAs for the ADAR ("radRNAs") to the subject, wherein the ADAR based RNA editing system specifically edits a point mutation in an RNA sequence encoding a gene.

[0202] The tRNA based protein editing system may comprise endogenous modified tRNA and/or orthogonal tRNA in order to prevent off target editing of proteins. In this regard, systems for the control of these tRNA are disclosed herein below.

[0203] The adRNA architecture for use in the ADAR based RNA editing system is relatively simple, comprising a RNA targeting domain, complementary to the target and, optionally, one or two recruiting domains (also referred to as aptamers) that recruit RNA binding domains of various proteins. The optional recruiting domains are positioned at the 5' and/or 3' ends of the RNA targeting domain. A schematic of adRNA bound to its mRNA target is provided in **FIG. 23C.** In some embodiments, the adRNA features an A-C mismatch, which prompts editing function of the ADAR. A similar framework can be used to target pre-mRNA, prior to intron processing by adapting the scaffold to target the pre-mRNA present in the nucleus. This approach is taken in the non-limiting exemplary methods involving OTC deficiency - involving a splice site mutation, whereas an mRNA editing approach is taken in the non-limiting exemplary methods involving dystrophin deficiency - involving a nonsense mutation.

[0204] Applicants tested a series of scaffolds, shown in **FIG. 19C,** to recruit RNA binding domains of the ADARs. The sequences provided in the figure represent the recruiting domain and the italicized Ns represent the nucleotides complimentary to the target. The C is the mismatch that prompts the editing function. Sequences of varying length and mismatch position may be tested to determine the best adRNA for the desired target. For example, residues in the recruiting domain of the adRNAs generated by Applicants were modified as follows (5'-3'):

v1 : GGGTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACCA*NNNNNNNNNNNNNNNN*

v2 : GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCAC*NNNNNCNNNNNNNNNNNN*

v3 : GTGGAAGAGGAGAACAATATGCTAAA.TGTTGTTCTCGTCTCCCAC

v4 : GGGTGGAA!iAGGAGAACAATATGCTAAATGTTGTTCTCGTCTCCCACCT

v5 : GGTGAAGAGGAGAACAATATGCTAAATGTTGTTCTCGTCTCCACC

v6 : GGTGAAGAGGAGAACAATATGCTAAATGTTGTTCTCGTCTCCACC

v7 : GTGGAAGAGGAGAACAATAGGCTAAACGTTGTTCTCGTCTCCCAC

v8 : GGGTGGAAGAGGAGAACAATAGGCTAAACGTTGTTCTCGTCTCCCACCT

v9 : GGTGAA!iAGGAGAACAATAGGCTAAACGTTGTTCTGGTCTCCACC

v10 : GGTGAAGAGGAGAACAATAGGCTAAACGTTGTTCTCGTCTCCACC

v11 : GGTGTCGAGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCTCGACACC

vl2 : GGTGTCGAGAAGAGGAGAACAATATGCTAAATGTTGTTCTCGTCTCCTCGACACC

vl3 : GGTGTCGAGAA.GAG!iAGAACAATAGGCTAAACGTTGTTGTCGTGTCCTCGACACC

[0205] The structure of V2 after folding is provided as **FIG. 23D.** And the corresponding radRNAs were generated as follows:

TCCACCCTATGATATTGTTGTAAATCGTATAACAATATGATAAGGTGGG

CACCCTATGATATTGTTGTAAATCGTATAACAATATGATAAGGTG

CACCCTCTGCTCTTGTTGTAAATCGTATAACAAGAGGAGAAGGTG

TCCACCCTCTGCTCTTGTTGTAAATCGTATAACAAGAGGAGAAGGTGGG

CCACCTCTGCTCTTGTTGTAAATCGTATAACAAGAGGAGAAGTGG

CCACCTCTGCTCTTGTTGTAAATCGTATAACAAGAGGAGAAGTGG

CACCCTCTGCTCTTGTTGCAAATCGGATAACAAGAGGAGAAGGTG

TCCACCCTCTGCTCTTGTTGCAAATCGGATAACAAGAGGAGAAGGTGGG

CCACCTCTGCTCTTGTTGCAAATCGGATAACAAGAGGAGAAGTGG

CCACCTCTGCTCTTGTTGCAAATCGGATAACAAGAGGAGAAGTGG

CCACAGCTCCTCTGCTCTTGTTGCAAATCGGATAACAAGAGGAGAAGAGCTGTGG

CCACAGCTCCTCTGCTCTTGTTGTAAATCGTATAACAAGAGGAGAAGAGCTGTGG

CCACAGCTCCTATGATATTGTTGTAAATCGTATAACAATATGATAAGAGCTGTGG

[0206] A schematic of the resulting adRNA and radRNA pairings to the target mRNA is shown in **FIG. 16C.**

[0207] An alternative scaffold framework was also applied by Applicants using two ADAR recruiting domains (black font) on either side of the targeting domain while varying the position of the C mismatch in the targeting domain (italicized Ns).

TGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCAC*NNNNNNNNNNNNNNN*GTGGAATAGTATAACAATA TGCTAAATGTTGTTATAGTATCCCAC

[0208] These non-limiting exemplary scaffolds provide a template for the engineering of adRNA and radRNA for particular targets and may be optimized based on comparative efficacy studies carried out according to the exemplary methods disclosed herein.

[0209] In some embodiments, the ADAR based editing system further comprises ADAR1, ADAR2, the E488Q and E100Q mutants each thereof, a fusion protein comprising the catalytic domain of an ADAR and a domain which associates with an RNA hairpin motif, a fusion protein comprising the catalytic domain of an ADAR and a dead Cas9, or a fusion protein comprising the double stranded binding domain of an ADAR and an APOBEC. In further embodiments, the domain which associates with an RNA hairpin motif is selected from the group of an MS2 bacteriophage coat protein (MCP) and an N22 peptide. In some embodiments, the adRNA comprises one or more RNA hairpin motifs. In some embodiments, the one or more RNA hairpin motifs are selected from the group of an MS2 stem loop and a BoxB loop.

[0210] Not to be bound by theory, Applicants believe the double stranded RNA binding motif from ADARs may bind to several double stranded RNA sequences and could thus have possible off target effects. To avoid such effects, Applicants contemplate the use of exogenous protein domains to recognize RNA hairpin motifs in the adRNA. Both ADARl and ADAR2 consist of RNA binding domains and a catalytic domain that catalyzes the conversion of adenosine to inosine. The catalytic domain can be uncoupled from the RNA binding domain. Our aim is to achieve high editing efficiency of the targeted adenosine while reducing off target effects and thus are exploring alternative RNA binding domains. Applicants have fused the catalytic domain of the ADARl or ADAR2 to different RNA binding domains such as the MCP, N22 or a dead CjCas9 (or other RNA targeting CRISPRs such as from SaCas9, CRISPR-Casl3 etc.). Upon the addition of appropriate guide RNAs

(adRNAs), the fusion proteins are recruited to the target, further catalyzing an adenosine to inosine change. The dead CjCas9 (and other CRISPRs by extension) in this case basically serves as a RNA binding domain that can in turn be tethered to effectors.

[0211] The domains are fused to the ADAR catalytic domain to generate ADAR specifically targeting the particular adRNA comprising the RNA hairpin motifs. For example, Applicants have used a MS2 bacteriophage coat protein (MCP) fused to either the catalytic domain of ADARl or ADAR2 and their respective mutants E488Q and E1008Q, while using a MS2 stem loop on the RNA to recruit the fusion protein **(FIG. 23A).** Analogous to this system, Applicants have also utilized a N22 peptide fused to the catalytic domains of ADARl or ADAR2 (and their mutants) while making use of a boxB aptamer to recruit the fusion protein. Thus, one or two copies of ADAR may be recruited based on the addition of single or dual hairpins (MS2/BoxB loops) **(FIG. 23A).** PP7 hairpins are also contemplated for use in the same manner.

[0212] A non-limiting framework sequence for the recruitment of MCP-based fusion proteins, where the C mismatch may be varied within the targeting domain, is provided herein below (with the lower case letters representing those linkers that help stabilize the underlined hairpins):

Single recruiting domain (underlined):

NNNNNNNNNV NNNV NNNNggccAACATGAGGATCACCCATGT CTGC7GggCc

Two recruiting domains (underlined):

¾ACATGAGGATCACCCATGTc.NNNNNNNNNNNNNNNNNNNNAACATGAGGATCACCCATGT c

An analogous non-limiting framework sequence is provided for the N22-based fusion proteins:

Single recruiting domain (underlined):

NNNNNNNNNNNNNNNNNNNggccctgaagaagggcCc

Two recruiting domains (underlined):

ggGCCCTGAAGAAGGGCc cNNNNNNNNNNNNNNNNNNNN¾-.;GCCCTGAAGAAGGGCc c

[0213] Another approach is to recruitment domains in the adRNA associated with Cas9 and couple a dead Cas9 to the ADAR catalytic domain, thus, rendering the same effect of specific recruitment. A non-limiting framework sequence for the recruitment is provided for Cas9-based fusion proteins:

Psp dCasl3a recruitment (mismatch position can be varied)

CAACATTATCGGGGAGTTTTGACCTCCAAGGTGTTGi;i¾N^[illegible]

Cj dCas9 recruitment (mismatch position can be varied)

[illegible]gttttagtccctgaaaagggactaaaataaagagtttgcgggactctgcggggt tacaatcccctaaaaccgo tttttttt

[0214] APOBECs also have RNA editing function **(FIG. 23B).** Thus, they may be used in the alternative or in addition to the ADAR based editing system. For example, Applicants have created MCP/N22 peptide fusions with APOBECs to engineer targeted C->T RNA editing. In addition, Applicants have fused the double stranded RNA binding domains (dsRBD) of the ADAR2 with APOBECs as a result of which the APOBECs can be recruited by the adRNA.

[0215] The addition of Nuclear Localization Signals (NLS) to the fusion protein could help target nuclear RNA *(i.e.* pre-mRNA) while addition of Nuclear Export Signals (NES) to the fusion protein could help target cytoplasmic RNA in any of the embodiments disclosed herein. This method is useful when editing for splice site mutations, which result in incorrect processing of introns in the pre-mRNA and, thus, results in incorrect mRNA for translation. OTC deficiency is example where targeting pre-mRNA with adRNA scaffolds can be useful, since the majority of aberrant OTC expression comes from the splice site mutation resulting in a truncated OTC protein. Further addition of RNA localization tags to the adRNA will enable targeting RNA in specific cellular compartments.

[0216] In further embodiments where the adRNA comprises one or more RNA hairpin motifs, the one or more RNA hairpin motifs are stabilized by replacing A-U with G-C. In some embodiments, the adRNA is stabilized through the incorporation of one or more of 2'-O-methyl, 2**'-0**-methyl 3'phosphorothioate, or 2**'-0**-methyl 3'thioPACE at either or both termini of the adRNA.

[0217] More generally, can be appreciated that the RNA targeting domains of adRNAs are designed such that they are complementary to the target mRNA while containing C mismatch at the position of the target adenosine. The recruiting domains of the adRNA are constant. BY way of non-limiting example:

Example target: OTC mRNA (mutation underlined)

5'-AAAGTCTCACAGACACCGCTCAGTTTGTAAAACTTTTCTTC- 3'

adRNA v2 (targeting domain length 20bp, mismatch position after 6 bases):

5'-AAAGTCTCACAGACACCGCTCAGTTTGTAAAACTTTTCTTC-
3'

5'-GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCA^ TCTGTGGCGAGCAAACA-3'

adRNA v2 (targeting domain length 21bp, mismatch position after 6 bases):

5'-AAAGTCTCACAGACACCGCTCAGTTTGTAAAACTTTTCTTC-
3'

5'-GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCA^ TCTCTGTGGCGAGCAAACA-3'

radRNA v2 (targeting domain length 20bp, mismatch position after 6 bases):

3'-CTTCTTTTCAAAATGTTTGACTCGCCACAGACACTCTGAAA- 5'

5'-iy GTTTTACi AACAA GCGCACCCTATGATATTGTTGTAAATCGTATAACAATATGATAAGGTG-3'

adRNA dual (targeting domain length 20bp, mismatch position after 5, 14 bases):

3'-CTTCTTTTCAAAATGTTTGACTCGCCACAGACACTCTGAAA- 5'

5'-
TGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCAC¾ AACAAGCGGTGTCTGTGGTGGAATAGTATAACAATA
TGCTAAATGTTGTTATAGTATCCCAC- 3'

adRNA MS2 (targeting domain length 20bp, mismatch position after 14 bases)

3'-CTTCTTTTCAAAATGTTTGACTCGCCACAGACACTCTGAAA- 5'

5'-CAAACAAGCGTGTCTGTGg aa**AACATGAGGATCACCCATGT**TGCAGggcc-3▪

adRNA MS2 dual (targeting domain length 20bp, mismatch position after 5, 14 bases)

3'- CTTCTTTTCAAAATGTTTGACTCGCCACAGACACTCTGAAA- 5'

5'-**aACATGAGGATCACCCATGTc**CAAAAGGAGCGGTGTCTGTG**laACATGAGGATCACCCATGTc**-3'

adRNA BoxB (targeting domain length 20bp, mismatch position after 14 bases)

3'- CTTCTTTTCAAAATGTTTGACTCGCCACAGACACTCTGAAA- 5'

5'-CAAAAGGAGCGGTGTCTGTG**gggccctgaagaagggc:** cc-3'

adRNA BoxB dual (targeting domain length 20bp, mismatch position after 5, 14 bases)

3'- CTTCTTTTCAAAATGTTTGACTCGCCACAGACACTCTGAAA- 5'

5'-**ggGCCCTGAAGAAGGGCcc** CAAAAGGAGCGGTGTCTGTG**ggGCCCTGAAGAAGGGCcc** -3'

[0218] A coordinate or alternate approach to preventing off-target effects is to make use of endogenous ADAR. ADAR2 is highly expressed in tissues such as the brain, lung and spleen while ADAR1 is ubiquitously expressed with general expression levels being higher than ADAR1. Thus, Applicants propose two avenues in order to engineer RNA editing by endogenous ADARs. First, ADAR1 expression can be stimulated by molecules such as interferons, *e.g.*, interferon a. Second, scaffolds may be engineered specifically for recruiting ADAR1 and are carrying out experiments with the vl-vl3 scaffolds as well as some chemically modified scaffolds disclosed herein above. Making use of the endogenous ADARs as opposed to overexpression could help limit the off-target effects.

Recombinant Expression Systems and Vectors

[0219] Aspects of the disclosure relate to vectors and recombinant expression systems.

[0220] For example, some aspects relate to a vector encoding one or more tRNA having an anticodon sequence that recognizes a codon comprising a point mutation in an RNA sequence encoding a protein, optionally wherein the point mutation results in a premature stop codon. In some embodiments, the point mutation results in a nonsense mutation having the DNA sequence TAA and the RNA sequence UAA. In some embodiments, the tRNA is an endogenous tRNA with a modified anticodon stem recognizing the codon comprising the point mutation. In further embodiments, the tRNA is charged with a serine. In some

embodiments, the tRNA is an orthogonal tRNA charged with a non-canonical amino acid. In further embodiments, the vector further comprises a corresponding tRNA synthetase. In some embodiments, the corresponding synthetase is E. coli Glutaminyl-tRNA synthetase. In some embodiments involving an orthogonal tRNA, the non-canonical amino acid is pyrrolysine. In some embodiments, the vector encodes two tRNA having an anticodon sequence that recognizes the codon comprising the point mutation. In some embodiments, the vector is an AAV vector, optionally an AAV8 vector. In some embodiments, the protein is dystrophin.

[0221] Further aspects relate to a recombinant expression system comprising one or more vectors encoding an ADAR based RNA editing system comprising one or more forward guide RNAs for the ADAR ("adRNAs") and one or more corresponding reverse guide RNAs for the ADAR ("radRNAs") to the subject, wherein the ADAR based RNA editing system specifically edits a point mutation in an RNA sequence encoding a protein. In some embodiments, the point mutation results in a nonsense mutation, optionally a premature stop codon, having the DNA sequence TAA and the RNA sequence UAA. In some embodiments, the ADAR based RNA editing system converts UAA to UIA and, optionally, further UIA to UII. In some embodiments, the ADAR based RNA editing system converts UAA to UAL In some embodiments, the point mutation results in a splice site or missense mutation having the DNA sequence CAG and the RNA sequence CAG. In some embodiments, the ADAR based RNA editing system converts CAG to CIG. In further embodiments, the one or more vector further encodes a tRNA that targets an amber codon. In some embodiments, the ADAR based editing system further comprises ADARl, ADAR2, the E488Q and E100Q mutants each thereof, a fusion protein comprising the catalytic domain of an ADAR and a domain which associates with an RNA hairpin motif, a fusion protein comprising the catalytic domain of an ADAR and a dead Cas9, or a fusion protein comprising the double stranded binding domain of an ADAR and an APOBEC. In further embodiments, the domain which associates with an RNA hairpin motif is selected from the group of an MS2 bacteriophage coat protein (MCP) and an N22 peptide. In some embodiments, the adRNA comprises one or more RNA hairpin motifs. In some embodiments, the one or more RNA hairpin motifs are selected from the group of an MS2 stem loop and a BoxB loop and/or are stabilized by

replacing A-U with G-C. In some embodiments, the adRNA is stabilized through the incorporation of one or more of 2'-0-methyl, 2'-0-methyl 3'phosphorothioate, or 2'-0-methyl 3'thioPACE at either or both termini of the adRNA.

[0222] In general methods of packaging genetic material such as RNA into one or more vectors is well known in the art. For example, the genetic material may be packaged using a packaging vector and cell lines and introduced via traditional recombinant methods.

[0223] In some embodiments, the packaging vector may include, but is not limited to retroviral vector, lentiviral vector, adenoviral vector, and adeno-associated viral vector (optionally AAV8). The packaging vector contains elements and sequences that facilitate the delivery of genetic materials into cells. For example, the retroviral constructs are packaging plasmids comprising at least one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required to package a replication incompetent retroviral vector, and for producing virion proteins capable of packaging the replication-incompetent retroviral vector at high titer, without the production of replication-competent helper virus. The retroviral DNA sequence lacks the region encoding the native enhancer and/or promoter of the viral 5' LTR of the virus, and lacks both the psi function sequence responsible for packaging helper genome and the 3' LTR, but encodes a foreign polyadenylation site, for example the SV40 polyadenylation site, and a foreign enhancer and/or promoter which directs efficient transcription in a cell type where virus production is desired. The retrovirus is a leukemia virus such as a Moloney Murine Leukemia Virus (MMLV), the Human Immunodeficiency Virus (HIV), or the Gibbon Ape Leukemia virus (GALV). The foreign enhancer and promoter may be the human cytomegalovirus (HCMV) immediate early (IE) enhancer and promoter, the enhancer and promoter (U3 region) of the Moloney Murine Sarcoma Virus (MMSV), the U3 region of Rous Sarcoma Virus (RSV), the U3 region of Spleen Focus Forming Virus (SFFV), or the HCMV IE enhancer joined to the native Moloney Murine Leukemia Virus (MMLV) promoter.

[0224] The retroviral packaging plasmid may consist of two retroviral helper DNA sequences encoded by plasmid based expression vectors, for example where a first helper

sequence contains a cDNA encoding the gag and pol proteins of ecotropic MMLV or GALV and a second helper sequence contains a cDNA encoding the env protein. The Env gene, which determines the host range, may be derived from the genes encoding xenotropic, amphotropic, ecotropic, polytropic (mink focus forming) or 10A1 murine leukemia virus env proteins, or the Gibbon Ape Leukemia Virus (GALV env protein, the Human Immunodeficiency Virus env (gpl60) protein, the Vesicular Stomatitus Virus (VSV) G protein, the Human T cell leukemia (HTLV) type I and II env gene products, chimeric envelope gene derived from combinations of one or more of the aforementioned env genes or chimeric envelope genes encoding the cytoplasmic and transmembrane of the aforementioned env gene products and a monoclonal antibody directed against a specific surface molecule on a desired target cell. Similar vector based systems may employ other vectors such as sleeping beauty vectors or transposon elements.

[0225] The resulting packaged expression systems may then be introduced via an appropriate route of administration, discussed in detail with respect to the method aspects disclosed herein.

Compositions

[0226] Further aspects relate to a composition comprising any one or more of the vectors disclosed herein. In some embodiments, the composition further comprises an effective amount of an interferon to enhance endogenous ADAR1 expression. In still further embodiments, the interferon is interferon a.

[0227] Briefly, pharmaceutical compositions of the present disclosure including but not limited to any one of the claimed compositions may comprise a target cell population as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients.

[0228] Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the disclosure. Those skilled in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation.

[0229] Such compositions may also comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions of the present disclosure may be formulated for oral, intravenous, topical, enteral, and/or parenteral administration. In certain embodiments, the compositions of the present disclosure are formulated for intravenous administration.

[0230] Administration of the compositions can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. Suitable dosage formulations and methods of administering the agents are known in the art. In a further aspect, the cells and composition of the disclosure can be administered in combination with other treatments.

[0231] The vectors, recombinant expression systems, and/or compositions are administered to the host using methods known in the art. This administration of the compositions of the disclosure can be done to generate an animal model of the desired disease, disorder, or condition for experimental and screening assays.

[0232] Briefly, pharmaceutical compositions of the present disclosure including but not limited to any one of the claimed compositions may comprise one or more vectors or recombinant expression systems as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions of the present disclosure may be formulated for oral, intravenous, topical, enteral, and/or parenteral administration. In certain embodiments, the compositions of the present disclosure are formulated for intravenous administration.

[0233] Pharmaceutical compositions of the present disclosure may be administered in a manner appropriate to the disease, disorder, or condition to be treated or prevented. The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages may be determined by clinical trials.

Methods of Restoring Protein Expression

[0234] Aspects of the disclosure relate to methods of restoring protein expression.

[0235] For example, some aspects of the disclosure relate to a method for restoring expression of a protein comprising a point mutation in an RNA sequence encoding the protein in a subject in need thereof comprising administering a vector encoding one or more tRNA having an anticodon sequence that recognizes a codon comprising the point mutation to the subject, optionally wherein the point mutation results in a premature stop codon. In some embodiments, the point mutation results in a nonsense mutation having the DNA sequence TAA and the RNA sequence UAA. In some embodiments, the tRNA is an endogenous tRNA with a modified anticodon stem recognizing the codon comprising the point mutation. In further embodiments, the tRNA is charged with a serine. In some embodiments, the tRNA is an orthogonal tRNA charged with a non-canonical amino acid. In further embodiments, the vector further comprises a corresponding tRNA synthetase. In some embodiments, the corresponding synthetase is E. coli Glutaminyl-tRNA synthetase. In some embodiments involving an orthogonal tRNA, the non-canonical amino acid is pyrrolysine. In further embodiments, the pyrrolysine is introduced in the diet of the subject. In some embodiments, the vector encodes two tRNA having an anticodon sequence that recognizes the codon comprising the point mutation. In some embodiments, the protein is dystrophin.

[0236] Other aspects relate to a recombinant expression system comprising one or more vectors encoding an ADAR based RNA editing system comprising one or more forward guide RNAs for the ADAR ("adRNAs") and one or more corresponding reverse guide RNAs for the ADAR ("radRNAs") to the subject, wherein the ADAR based RNA editing system specifically edits a point mutation in an RNA sequence encoding a protein. In some

embodiments, the point mutation results in a nonsense mutation, optionally a premature stop codon, having the DNA sequence TAA and the RNA sequence UAA. In some embodiments, the ADAR based RNA editing system converts UAA to UIA and, optionally, further UIA to UII. In some embodiments, the ADAR based RNA editing system converts UAA to UAL In some embodiments, optionally those involving nonsense or missense mutations, the RNA targeted in mRNA. In further embodiments, the one or more vector further encodes a tRNA that targets an amber codon. In some embodiments, the protein is dystrophin. In some embodiments, the point mutation results in a splice site or missense mutation having the DNA sequence CAG and the RNA sequence CAG. In some embodiments, the ADAR based RNA editing system converts CAG to CIG. In some embodiments, optionally those involving splice site mutations, the RNA targeted is pre-mRNA. In some embodiments, the ADAR based editing system further comprises ADARl, ADAR2, the E488Q and E100Q mutants each thereof, a fusion protein comprising the catalytic domain of an ADAR and a domain which associates with an RNA hairpin motif, a fusion protein comprising the catalytic domain of an ADAR and a dead Cas9, or a fusion protein comprising the double stranded binding domain of an ADAR and an APOBEC. In further embodiments, the domain which associates with an RNA hairpin motif is selected from the group of an MS2 bacteriophage coat protein (MCP) and an N22 peptide. In some embodiments, the adRNA comprises one or more RNA hairpin motifs. In some embodiments, the one or more RNA hairpin motifs are selected from the group of an MS2 stem loop and a BoxB loop and/or are stabilized by replacing A-U with G-C. In some embodiments, the adRNA is stabilized through the incorporation of one or more of 2'-0-methyl, 2'-0-methyl 3'phosphorothioate, or 2'-0-methyl 3'thioPACE at either or both termini of the adRNA.

[0237] In either case, the assessment of whether protein expression is "restored" is achieved through any means of protein quantification when compared to a baseline. The baseline may optionally be calculated based on a prior level in the subject or as the normal level in the population, adjusted for the subjects age, ethnicity, and other relevant demographic information. Techniques of quantifying protein expression are well known in the art and may, optionally, utilize a control or a threshold value for comparison to the baseline value. Methods known in the art for such studies include but are not limited to qRTPCR,

ELISA,Western blot, protein immunostaining, spectroscopy and/or spectrometry based methods, and other assays typically conducted to determine the amount of protein expression in a sample from the subject. Alternatively, the "restoration" effect may be determined based on a clinical outcome. For example, aberrant dystrophin levels are linked to muscular dystrophy symptoms. Thus, the restoration of expression may be outwardly determined based on clinical signals such as a reduction or reversal of these symptoms. For dystrophin, improvement in muscle strength can be one such indicator. Thus, physicians may carry out strength measurements to determine outcome. Another example is ornithine transcarbamylase (OTC); aberrant OTC levels are a result of a rare X-linked genetic disorder resulting in excessive accumulation of ammonia in the blood (due to nitrogen accumulation). Thus, a relevant clinical outcome would be a decrease in ammonia in a biological sample, such as blood or urine. Similarly, clinical signals associated with and expression of proteins downstream of the protein of interest may be relevant indicators of "restoration" where the protein of interest is involved in a particular pathway.

Methods of Treatment

[0238] Point mutations are implicated in a number of diseases, disorders, and conditions. Non-limiting examples are provided in **Table 1** below.

Table 1

Protein/Disease, Disorder, or Condition	Associated Point Mutation
G to A point mutations or premature stop codons	
Dihydropyrimidine dehydrogenase deficiency	NM_0001 10.3(DPYD):c.l905+lG>A
Noonan syndrome	NM_005633.3(SOSl):c.2536G>A (p.Glu846Lys)
Lynch syndrome	NM_00025 1.2(MSH2):c.212-lG>A
Breast-ovarian cancer, familial 1	NM_007294.3(BRCAl):c.963G>A (p.=)
Cystic fibrosis	NM_000492.3(CFTR):c.57G>A (p.=)
Anemia, due to G6PD deficiency	NM_000402.4(G6PD):c.292G>A (p.Val98Met)
AVPR2 Nephrogenic diabetes insipidus, X-linked	NM_000054.4(AVPR2):c.878G>A (p.=)
FANCC Fanconi anemia, complementation group C	NM_000054.4(AVPR2):c.878G>A (p.=)
FANCC Fanconi anemia, complementation group C	NM_000136.2(FANCC):c.l517G>A (p.=)
IL2RG X-linked severe combined immunodeficiency	NM_000206.2(IL2RG):c.710G>A (p.=)
F8 Hereditary factor VIII deficiency disease	NM_000132.3(F8):c.3 144G>A (p.=)
LDLR Familial hypercholesterolemia	NM_000527.4(LDLR):c.l449G>A (p.=)
CBS Homocystinuria due to CBS deficiency	NM_000071.2(CBS):c.l62G>A (p.=)
HBB betaThalassemia	NM_0005 18.4(HBB):c.1 l4G>A (p.=)
ALDOB Hereditary fructosuria	NM_000035.3(ALDOB):c.888G>A (p.=)
DMD Duchenne muscular dystrophy	NM_004006.2(DMD):c.3747G>A (p.=)
SMAD4 Juvenile polyposis syndrome	NM_005359.5(SMAD4):c.906G>A (p.=)
BRCA2 Familial cancer of breast|Breast-ovarian cancer, familial 2	NM_000059.3(BRCA2):c.582G>A (p.=)
GRIN2A Epilepsy, focal, with speech disorder and with or without mental retardation	NM_000833.4(GRIN2A):c.3813G>A (p.=)
SCN9A Indifference to pain, congenital, autosomal recessive	NM_002977.3(SCN9A):c.269 1G>A (p.=)
TARDBP Amyotrophic lateral sclerosis type 10	NM_007375.3(TARDBP):c.943G>A (p.Ala3 15Thr)
CFTR Cystic fibrosis|Hereditary pancreatitis|not provided|ataluren response - Efficacy	NM_000492.3(CFTR):c.3846G>A (p.=)
UBE3A Angelman syndrome	NM_130838.l(UBE3A):c.2304G>A (p.=)
SMPDl Niemann-Pick disease, type A	NM_000543.4(SMPDl):c.l68G>A (p.=)
USH2A Usher syndrome, type 2A	NM_206933.2(USH2A):c.9390G>A (p.=)
MEN1 Hereditary cancer-predisposing syndrome	NM_130799.2(MENl):c.l269G>A (p.=)
C8orf37 Retinitis pigmentosa 64	NM_177965.3(C8orf37):c.555G>A (p.=)
MLHl Lynch syndrome	NM_000249.3(MLHl):c.l998G>A (p.=)
TSC2 Tuberous sclerosis 2|Tuberous sclerosis syndrome 46	NM_000548.4(TSC2):c.2108G>A (p.=)
NFl Neurofibromatosis, type 1	NM_000267.3(NFl):c.7044G>A (p.=)
MSH6 Lynch syndrome	NM_000179.2(MSH6):c.3020G>A (p.=)
SMN1 Spinal muscular atrophy, type II|Kugelberg-Welander disease	NM_000344.3(SMNl):c.305G>A (p.=)
SH3TC2 Charcot-Marie-Tooth disease, type 4C	NM_024577.3(SH3TC2):c.920G>A (p.=)
DNAH5 Primary ciliary dyskinesia	NM_00 1369.2(DNAH5):c.8465G>A (p.=)
MECP2 Rett syndrome	NM_004992.3(MECP2):c.3 11G>A (p.=)
ADGRV1 Usher syndrome, type 2C	NM_032 119.3(ADGRVl):c.7406G>A (p.=)
AHIl Joubert syndrome 3	NM_01765 1.4(AHIl):c.2174G>A (p.=)
PRKN Parkinson disease 2	NM_004562.2(PRKN):c.l358G>A (p.=)
COL3A1 Ehlers-Danlos syndrome, type 4	NM_000090.3(COL3Al):c.3833G>A (p.=)
BRCA1 Familial cancer of breast|Breast-ovarian	NM_007294.3(BRCAl):c.55 11G>A (p.=)

cancer, familial 1	
MYBPC3 Primary familial hypertrophic cardiomyopathy	NM_000256.3(MYBPC3):c.3293G>A (p.Trpl098Ter)
APC Familial adenomatous polyposis 1	NM_000038.5(APC):c. l262G>A (p.Trp421Ter)
BMPR2 Primary pulmonary hypertension	NM_001204.6(BMPR2):c.893G>A (p.W298*)
T to C point mutations	
Wilson disease	NM_000053.3(ATP7B):c.3443T>C (p.Ilel l48Thr)
Leukodystrophy, hypomyelinating, 2	NM_020435.3(GJC2):c.857T>C (p.Met286Thr)
Alport syndrome, X-linked recessive	NM_000495.4(COL4A5):c.438+2T>C
Leigh disease	NC_012920.1:m.9478T>C
Gaucher disease, type 1	NM_001005741.2(GBA):c.751T>C (p.Tyr251His)
Renal dysplasia, retinal pigmentary dystrophy, cerebellar ataxia and skeletal dysplasia	NM_014714.3(IFT140):c.4078T>C (p.Cysl360Arg)
Marfan syndrome	NM_000138.4(FBNl):c.3793T>C (p.Cysl265Arg)
Deficiency of UDPglucose-hexose-l-phosphate uridylyltransferase	NM_000155.3(GALT):c.482T>C (p.Leul61Pro)
Familial hypercholesterolemia	NM_000527.4(LDLR):c.694+2T>C
Episodic pain syndrome, familial, 3	NM_001287223.1(SCN11A):c.1142T>C (p.Ile381Thr)
Navajo neurohepatopathy	NM_002437.4(MPV17):c.l86+2T>C
Congenital muscular dystrophy, LMNA-related	NM_170707.3(LMNA):c.l1139T>C (p.Leu380Ser)
Hereditary factor VIII deficiency disease	NM_000132.3(F8):c.5372T>C (p.Metl791Thr)
Insulin-dependent diabetes mellitus secretory diarrhea syndrome	NM_014009.3(FOXP3):c.970T>C (p.Phe324Leu)
Hereditary factor IX deficiency disease	NM_000133.3(F9):c.1328T>C (p.Ile443Thr)
Familial cancer of breast, Breast-ovarian cancer, familial 2, Hereditary cancerpredisposing syndrome	NM_000059.3(BRCA2):c.316+2T>C
Cardiac arrhythmia	NM_000238.3(KCNH2):c.1945+6T>C
Tangier disease	NM_005502.3(ABCA1):c.4429T>C (p.Cysl477Arg)
Dilated cardiomyopathy 1AA	NM_001103.3(ACTN2):c.683T>C (p.Met228Thr)
Mental retardation 3, X-linked	NM_005334.2(HCFC1):c.-970T>C
Limb-girdle muscular dystrophy, type 2B	NM_003494.3(DYSF):c.1284+2T>C
Macular dystrophy, vitelliform, 5	NM_016247.3(IMPG2):c.370T>C (p.Phel24Leu)
Retinitis pigmentosa	NM_000322.4(PRPH2):c.736T>C (p.Tϕ246Arg)

[0239] Further non-limiting examples include Ornithine Transcarbamylase Deficiency, Nougaret night blindness, Usher syndrome, Atrial Fibrillation, Duchenne Muscular

Dyrstrophy, Wilson disease, hereditary tyrosinemia, and some cancers carrying a A -> G mutation in genes such as B-catenin.

[0240] Thus, aspects of this disclosure relate to the treatment of certain diseases, disorders, and conditions involving point mutations.

[0241] For example, some method aspects relate to a treating a disease, disorder, or condition characterized by the presence of a point mutation in an RNA sequence encoding a protein associated with the disease, disorder, or condition in a subject in need thereof comprising administering a vector encoding one or more tRNA having an anticodon sequence that recognizes a codon comprising the point mutation to the subject, optionally wherein the point mutation results in a premature stop codon. In some embodiments, the point mutation results in a nonsense mutation having the DNA sequence TAA and the RNA sequence UAA. In some embodiments, the tRNA is an endogenous tRNA with a modified anticodon stem recognizing the codon comprising the point mutation. In further embodiments, the tRNA is charged with a serine. In some embodiments, the tRNA is an orthogonal tRNA charged with a non-canonical amino acid. In further embodiments, the vector further comprises a corresponding tRNA synthetase. In some embodiments, the corresponding synthetase is E. coli Glutaminyl-tRNA synthetase. In some embodiments involving an orthogonal tRNA, the non-canonical amino acid is pyrrolysine. In further embodiments, the pyrrolysine is introduced in the diet of the subject. In some embodiments, the vector encodes two tRNA having an anticodon sequence that recognizes the codon comprising the point mutation. In some embodiments, the disease, disorder, or condition is selected from the group consisting of the diseases, disorders, and conditions listed in **Table 1,** optionally characterized by the presence of a nonsense mutation and/or a premature stop codon. In some embodiments, the protein is dystrophin. In further embodiments, the disease, disorder, or condition is muscular dystrophy. In still further embodiments, the disease disorder or condition is Duchenne muscular dystrophy.

[0242] Additional method aspects relate to a method of treating a disease, disorder, or condition by the presence of a point mutation in an RNA sequence encoding a protein associated with the disease, disorder, or condition in a subject in need thereof comprising

administering one or more vectors encoding an ADAR based RNA editing system comprising one or more forward guide RNAs for the ADAR ("adRNAs") and one or more corresponding reverse guide RNAs for the ADAR ("radRNAs") to the subject, wherein the ADAR based RNA editing system specifically edits the point mutation. In some embodiments, the point mutation results in a nonsense mutation, optionally a premature stop codon, having the DNA sequence TAA and the RNA sequence UAA. In some embodiments, the ADAR based RNA editing system converts UAA to UIA and, optionally, further UIA to UII. In some embodiments, the ADAR based RNA editing system converts UAA to UAL In some embodiments, optionally those involving nonsense or missense mutations, the RNA targeted in mRNA. In further embodiments, the one or more vector further encodes a tRNA that targets an amber codon. In some embodiments, the protein is dystrophin. In some embodiments, the point mutation results in a splice site or missense mutation having the DNA sequence CAG and the RNA sequence CAG. In some embodiments, the ADAR based RNA editing system converts CAG to CIG. In some embodiments, optionally those involving splice site mutations, the RNA targeted is pre-mRNA. In some embodiments, the ADAR based editing system further comprises ADARl, ADAR2, the E488Q and E100Q mutants each thereof, a fusion protein comprising the catalytic domain of an ADAR and a domain which associates with an RNA hairpin motif, a fusion protein comprising the catalytic domain of an ADAR and a dead Cas9, or a fusion protein comprising the double stranded binding domain of an ADAR and an APOBEC. In further embodiments, the domain which associates with an RNA hairpin motif is selected from the group of an MS2 bacteriophage coat protein (MCP) and an N22 peptide. In some embodiments, the method further comprises administering an effective amount of an interferon to enhance endogenous ADARl expression. In still further embodiments, the interferon is interferon a. In some embodiments, the adRNA comprises one or more RNA hairpin motifs. In some embodiments, the one or more RNA hairpin motifs are selected from the group of an MS2 stem loop and a BoxB loop and/or are stabilized by replacing A-U with G-C. In some embodiments, the adRNA is stabilized through the incorporation of one or more of 2'-0-methyl, 2'-0-methyl 3'phosphorothioate, or 2'-0-methyl 3'thioPACE at either or both termini of the adRNA. In some embodiments, the disease, disorder, or condition is selected from the group consisting of the diseases, disorders, and conditions listed in **Table 1**. In further

embodiments, the protein is dystrophin and the disease, disorder, or condition is muscular dystrophy. In still further embodiments, the disease disorder or condition is Duchenne muscular dystrophy.

[0243] An ordinary skilled artisan will appreciate that the doses and route of administration employed in these methods may vary based on the subject and the disease, disorder, or condition to be treated. Based on knowledge in the art such suitable doses and routes may be selected based on the subject's age, ethnicity, and other relevant demographic factors.

Kits

[0244] In one particular aspect, the present disclosure provides kits for performing any of the methods disclosed herein as well as instructions for carrying out the methods of the present disclosure and/or administering the vectors, recombinant expression systems, and compositions disclosed herein.

[0245] The kit can also comprise agents necessary for the preservation of those components comprised therein, e.g., a buffering agent, a preservative or a protein-stabilizing agent. The kit can further comprise components necessary for detecting the detectable-label, e.g., an enzyme or a substrate. The kit can also contain a control sample or a series of control samples, which can be assayed and compared to the test sample. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit. The kits of the present disclosure may contain a written product on or in the kit container. The written product describes how to use the reagents contained in the kit.

[0246] As amenable, these suggested kit components can be packaged in a manner customary for use by those of skill in the art. For example, these suggested kit components may be provided in solution or as a liquid dispersion or the like.

Examples

[0247] The following examples are non-limiting and illustrative of procedures which can be used in various instances in carrying the disclosure into effect. Additionally, all reference disclosed herein are incorporated by reference in their entirety.

Example 1 - Design of tRNA constructs

[0248] The tRNA constructs were designed along the lines of the schematics in **Figure 1** to recognize the nonsense mutation TAA. Both modified endogenous and orthogonal tRNA were generated. The constructs were validated *in vitro* using a GFP harboring nonsense mutation. It was determined that two copies of the tRNA should be include in each AAV vector for both modified endogenous and orthogonal tRNAs. MbPyl sythetase was selected for use with the orthogonal tRNA. The AAV vectors were generated comprising the tRNA and GFP (as well as the synthetase, where orthogonal tRNA was used). The sequences used in these constructs are provided in the Sequence Listing above.

Example 2 - Restoration of full length dystrophin in mdx mice

[0249] The anticodon stem of the human serine tRNA is modified such that it recognizes the nonsense codon (TAA). No endogenous tRNA can recognize a stop codon and translation terminates when the ribosome reaches a stop codon. Mdx mice bear a nonsense mutation (TAA) in the gene coding for dystrophin as a result of which they lack full length dystrophin expression. AAVs are used to deliver two copies of the modified tRNAs into the mouse muscle which in turn allows for the stop codon readthrough enabling the expression of full length dystrophin.

[0250] The calf muscles of mdx mice were injected with 1E12 particles of AAV8 carrying 2 copies of the modified serine tRNA and a GFP gene. These mice were then sacrificed after a month and the calf muscles were harvested. The muscles were sectioned and stained with an antibody against dystrophin. A clear restoration of dystrophin expression was noticed. In addition, the muscle morphology improved too.

[0251] Applicants have, thus, demonstrated activity of our vectors in vitro using a GFP gene harboring a stop codon. In addition Applicants have demonstrated restoration of dystrophin expression in mdx mouse muscles. Within a span of one month after injecting the mdx mice with AAVs carrying two copies of the serine tRNA, Applicants observed restoration of dystrophin expression in the calf muscle via Immunohistochemistry. Applicants also noted an improved muscle morphology.

[0252] This experiment is repeated with the orthogonal tRNA, introducing the pyrrolysine through the mouse feed, and is again replicated with both tRNAs in a larger population of mice.

Example 3 - Diet regulable production of therapeutic proteins

[0253] Applicants aim at achieving on-demand, in vivo production of therapeutics such as (i) insulin; (ii) neutralizing antibodies for viruses (e.g. HIV, HCV, HPV, influenza) and bacteria (e.g. staph aureus; drug resistant strains) by the skeletal muscle.

[0254] The desired transgenes are delivered to the muscle via AAVs (or lentiviruses) along with an orthogonal tRNA/tRNA synthetase pair. These transgenes contain a premature termination codon (stop codon) that will prevent the full length protein from being expressed. For an on demand synthesis of the therapeutics, an appropriate unnatural amino acid is consumed in the diet, which in turn is incorporated by the orthogonal tRNA/tRNA synthetase at the premature termination site, enabling synthesis of the desired therapeutics.

Example 4 - ADAR2 based RNA editing

[0255] Applicants suspected that ADAR2 (adenosine deaminase that acts on RNA) to correct mutations at the mRNA level. Applicants used Adeno-Associated Viruses to deliver the ADAR2 and a adRNA (forward ADAR2 guide) or radRNA (reverse ADAR2 guide) that direct the enzyme to the mutation in an attempt to restore the expression of dystrophin in the mdx mouse models of DMD, by editing the nonsense mutation. Applicants also applied this technology to the mouse model of the metabolic disorder Ornithine Transcarbamylase (OTC) deficiency.

[0256] As compared to nucleases, ADARs make site specific Adenosine to Inosine (A->I) changes in the mRNA with Inosine being read as a Guanosine (G) during translation and are thereby safe from creating permanent off target effects. Also, since they make edits at the mRNA level, the altered proteins are expressed only transiently. The use of nucleases in adult OTC-deficient mice led to large deletions that proved to be lethal to the animals. The use of ADARs might circumvent this problem. This could be a readily translatable solution for

several disorders characterized by point mutations. In addition, the origin of the ADAR2 is human, thereby minimizing the immune response generated by the body against it. Applicants also combine the idea of tRNA suppression with ADAR2 based RNA editing. In addition, Applicants designed hairpin loops (3' overlap) and toe-holds (5' overlap) that help improve the specificity of the adRNA/radRNA. Applicants also go on to optimize the lengths of the adRNA for efficient A->I editing as well as the ADAR2 recruiting domain of the adRNA/radRNA.

[0257] Existing studies have made use of nucleases such as Cas9 to delete the mutated region of the Dystrophin/OTC genes and replaced it with a functional copy, for the treatment of DMD or OTC deficiency caused by a point mutation. For DMD, existing therapies include the use of corticosteroids that delay the symptoms of the disorder. Other strategies include the premature stop codon readthrough by making use of drugs such as Ataluren or Gentamycin. Another strategy is that of exon skipping which results in a truncated protein, however able to reduce the severity of the DMD phenotype. Another approach is the delivery of a u-dystrophin gene. Clinical trials for OTC deficiency have been attempted making use of adenoviral vectors to deliver OTC cDNA in patients. Other avenues for treatment include use of sodium phenylbutyrate which helps increase the waste nitrogen excretion.

[0258] The use of ADAR2 as an engineered RNA editing enzyme has been demonstrated only *in vitro.*

[0259] Applicants utilized adRNAs and radRNAs comprised of two domains, one complementary to the target sequence and the other an ADAR2 recruiting domain. Applicants utilized AAVs to deliver these adRNAs/radRNAs along with the ADAR2 enzyme. Mdx mice bear a nonsense mutation (TAA) in the gene coding for dystrophin. Applicants packaged two copies of the adRNAs/radRNAs or a combination of adRNA/radRNA+tRNA along with the ADAR2 enzyme into the AAV and deliver it into the Tibialis Anterior (TA) muscle. Applicants utilized three alternative strategies to restore dystrophin expression:

(1) adRNAs/radRNAs that can edit both the adenosines in the 'TAA' to inosines;

(2) a sequential approach whereby the first adRNA/radRNA converts TAA -> TGA

and the next adRNA/radRNA converts it to TGG, restoring expression; and

(3) a combination of adRNAs/radRNAs and a tRNA whereby the adRNA/radRNA converts the TAA into TAG and the tRNA suppression of the amber codon (TAG) restores dystrophin expression.

[0260] Applicants also delivered two copies of the adRNA targeting the OTC G->A mutation in spf-ash mice along with the ADAR2 to the liver via retro-orbital injections.

[0261] The system works by editing an Adenosine to Inosine which is read as a Guanosine during translation. This can be used to correct point mutations as well as restore expression by editing premature stop codons. In **FIG. 6:** A. An Amber stop codon can be converted to a tryptophan codon by a single edit. B. Ochre stop codon - both edits made in a single step. C. Ochre stop codon - Sequential editing. D. Ochre stop codon - ADAR2 editing in combination with suppressor tRNA.

[0262] The following 10 steps represent the workflow to test these constructs:

1. Design and clone ADAR2 constructs - adRNA and radRNA.

2. In vitro validation of constructs using a GFP harboring a nonsense mutation.

3. Modification of constructs - decision to clone two copies of the adRNA/radRNA. Creation of vectors harboring one copy of a adRNA/radRNA and a copy of a serine suppressor tRNA.

4. Generation of AAV8 vectors carrying ADAR2 and adRNAs/radRNAs or suppressor tRNAs.

6. TA/Gastrocnemius injections of mdx mice - 1E12 particles of AAV8 carrying the ADAR2 and with adRNAs/radRNAs or suppressor tRNA were injected.

7. The mice were sacrificed 6 weeks after injections and the TA/gastrocnemimus were harvested. Immunohistochemistry performed to detect dystrophin. Some evidence of restoration of dystrophin.

8. qPCR, Western blots and NGS were carried out.

9. Vectors were optimized to improve efficiency. adRNA lengths varied, location of the edit varied.

10. Steps 4-8 repeated with optimized vectors.

[0263] Applicants designed adRNA and radRNAs against a premature stop codon in GFP and demonstrated robust restoration of expression **(FIG. 5).** For the ochre stop codon (TAA), two A->G edits are needed to restore expression. Applicants constructed a single ad/radRNA targeting both As or a two ad/radRNAs that target a single A in a sequential manner. Applicants also constructed an adRNA/radRNA+suppressor tRNA vector combining RNA editing with tRNA suppression.

[0264] In vitro RNA editing showed robust restoration of GFP expression after which AAVs bearing the ADAR2 and adRNA/radRNAs were generated to target the nonsense mutation in dystrophin in mdx mice.

[0265] The Tibialis Anterior (TA) or gastrocnemius muscles of mdx mice were injected with 1E12 particles of AAV8 carrying ADAR2 and two copies of the adRNA/radRNA or one copy of the adRNA/radRNA and a suppressor tRNA. These mice were sacrificed after 6 weeks and the appropriate muscles were harvested. The muscles were sectioned and stained with an antibody against dystrophin. Partial restoration of dystrophin expression was noticed.

[0266] In general, Applicants noticed a fractional restoration of dystrophin expression via Immunostaining. However, western blots and NGS did not show any evidence of editing/restoration of dystrophin expression.

[0267] Potential applications of the system include targeting point mutations for the treatment of disorders such as but not restricted to DMD, OTC deficiency, Wilson's disease and hereditary tysosinemia type 1. It could also be used to create alternate start codons, enabling the co-existence of a protein and its N-terminal truncated form.

Example 5 - ADAR Editing in mouse models

[0268] Genome engineering methodologies coupled with rapidly advancing synthetic biology toolsets are enabling powerful capabilities to perturb genomes for deciphering function, programming novel function, and repairing aberrant function. In particular, programmable DNA nucleases, such as meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR-Cas, have been widely used to engineer genomes across a range of organisms. Their use in gene therapy however poses at least three major challenges: one, the efficiency of homologous recombination versus non-homologous end joining is typically low, particularly in post-mitotic cells that comprise the vast majority of the adult body; two, an active nuclease always poses the threat of introducing permanent off-target mutations, thus presenting formidable challenges in both engineering exquisite nuclease specificity without compromising activity, as well as ensuring tight regulation of the nuclease dose and duration in target cells; and three, prevalent programmable nucleases are of prokaryotic origin or bear domains that are of non-human origin raising a significant risk of immunogenicity in *in vivo* therapeutic applications. The recent advent of base editing approaches is opening an exciting alternative strategy for gene targeting, but demonstrated approaches rely on CRISPR-Cas systems that are of prokaryotic origin. Thus for genomic mutations that lead to alteration in protein function, such as in disease causing gene mutations, approaches to instead directly target RNA would be highly desirable. Leveraging the aspect that single-stranded RNA as compared to double-stranded DNA, is generally more accessible to oligonucleotide mediated targeting without a need for additional enabling proteins, and building on the advances in tRNA mediated codon suppression and genetic code expansion, as well as adenosine deaminase mediated RNA editing, Applicants have engineered and optimized an integrated platform for RNA targeting, and demonstrate its efficacy in *in vitro* and *in vivo* applications.

Vector design and construction

[0269] To construct the GFP reporters - GFP-Amber, GFP-Ochre and GFP-Opal, three gene blocks were synthesized with 'TAG', 'TAA' and 'TGA' respectively replacing the Y39 residue of the wild type GFP and were cloned downstream of a CAG promoter. One, two, or

four copies of the endogenous suppressor tRNAs were cloned into an AAV vector containing a human U6 and mouse U6 promoter. Pyrrolysyl tRNAs and adRNAs/radRNAs were similarly cloned into an AAV vector containing a human U6 and mouse U6 promoter along with a CMV promoter driving the expression of MbPylRS/MmPylRS/AcKRS or hADAR2 respectively.

Mammalian cell culture and transfection

[0270] All HEK 293T cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10% FBS and 1% Antibiotic- Antimycotic (Thermo Fisher) in an incubator at 37 °C and 5% $C0_2$ atmosphere. All *in vitro* transfection experiments were carried out in HEK 293T cells using the commercial transfection reagent Lipofectamine 2000 (Thermo Fisher). All *in vitro* suppression and editing experiments were carried out in 24 well plates using 500ng of reporter plasmid and lOOOng of the suppressor tRNA/aaRS plasmid or the adRNA/ADAR2 plasmid. Cells were transfected at 30% confluence. Cells were harvested 48 and 72 hours post transfection for quantification of suppression and editing respectively. The UAAs Nε-Boc-L-Lysine (Chemimpex) and Nε-Acetyl-L-Lysine (Sigma) were added to the media at the desired concentration before transfection.

Production of AAV vectors

[0271] Virus was prepared using the protocol from the Gene Transfer, Targeting and Therapeutics (GT3) core at the Salk Institute of Biological Studies (La Jolla, CA). AAV8 particles were produced using HEK 293T cells via the triple transfection method and purified via an iodixanol gradient. Confluency at transfection was about 80%. Two hours prior to transfection, DMEM supplemented with 10% FBS was added to the HEK 293T cells. Each virus was produced in 5 x 15 cm plates, where each plate was transfected with 7.5 ug of pXR-8, 7.5 of ug recombinant transfer vector, 7.5 ug of pHelper vector using PEI (lug/uL linear PEI in lxDPBS pH 4.5, using HC1) at a PELDNA mass ratio of 4:1. The mixture was incubated for 10 minutes at RT and then applied dropwise onto the cell media. The virus was harvested after 72 hours and purified using an iodixanol density gradient ultracentrifugation method. The virus was then dialyzed with 1 x PBS (pH 7.2) supplemented with 50 mM NaCl and 0.0001% of Pluronic F68 (Thermo Fisher) using 50kDA filters (Millipore), to a final

volume of ~1 mL and quantified by qPCR using primers specific to the ITR region, against a standard (ATCC VR-1616).

AAV-ITR-F: 5'-CGGCCTCAGTGAGCGA-3' and

AAV-ITR-R: 5'-GGAACCCCTAGTGATGGAGTT-3'.

RNA isolation and Next Generation Sequencing library preparation

[0272] RNA from gastrocnemius or TA muscles of *mdx* mice or livers of *spf* ^{sh} mice was extracted using the RNeasy Plus Universal Mini Kit (Qiagen), according to the manufacturer's protocol. Next generation sequencing libraries were prepared as follows. cDNA was synthesized using the Protoscript II First Strand cDNA synthesis Kit (New England Biolabs). Briefly, 500 ng of input cDNA was amplified by PCR with primers that amplify 150 bp surrounding the sites of interest using KAPA Hifi HotStart PCR Mix (Kapa Biosystems). PCR products were gel purified (Qiagen Gel Extraction kit), and further purified (Qiagen PCR Purification Kit) to eliminate byproducts. Library construction was done with NEBNext Multiplex Oligos for Illumina kit (NEB). 10 ng of input DNA was amplified with indexing primers. Samples were then pooled and loaded on an Illumina Miseq (150 single-end run) at 5nM concentrations. Data analysis was performed using CRISPResso.

Animal Experiments

[0273] AAV Injections: All animal procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, San Diego. All mice were acquired from Jackson labs. AAVs were injected into the gastrocnemius or TA muscle of *mdx* mice (6-10 weeks old) using 2.5E+12 vg/muscle. AAVs were injected into *spf* ^{sh} (10-12 weeks old) mice via retro-orbital injections using 3E+12 vg/mouse.

[0274] UAA administration: Mice were fed water containing 20 mg/ml Nε-Boc-L-Lysine (Chemimpex) for one month. Mice were also administered the 30 mg Nε-Boc-L-Lysine via IP injections, thrice a week.

Immunofluorescence

[0275] Harvested gastrocnemius or TA muscles were placed in molds containing OCT compound (VWR) and flash frozen in liquid nitrogen. 20 μιη sections were cut onto pre-treated histological slides. Slides were fixed using 4% Paraformaldehyde. Dystrophin was detected with a rabbit polyclonal antibody against the N-terminal domain of dystrophin (1:100, Abeam 15277) followed by a donkey anti-rabbit Alexa 546 secondary antibody (1:250, Thermo Fisher).

Statistical Analysis

[0276] All statistical analyses were performed using the software Graphpad Prism and p-values were computed by unpaired two-tailed t tests.

Results

[0277] Applicants focused first on establishing the system for targeting nonsense mutations. This was motivated by the fact that nonsense mutations are responsible for 11% of all described gene lesions causing inheritable human disease, and close to 20% of disease-associated single base substitutions that affect the coding regions of genes. Specifically, we explored two independent but complementary approaches to directly target nonsense mutations. First, Applicants focused on engineering robust nonsense codon suppression via suppressor tRNAs. Although the use of suppressor tRNAs for premature stop codon read-through of endogenous non-sense mutations has been attempted *in vivo* in mice, these prior studies relied only on plasmid delivery and the use of robust and optimized delivery formats was not explored. Additionally, the potential use of un-natural amino acid (UAA) based inducible *in vivo* suppression of a disease-causing endogenous nonsense mutation has not been explored either. Towards this, Applicants first modified the anticodon stems of serine, arginine and leucine tRNAs to create suppressor tRNAs targeting all three stop codons, amber, opal and ochre, and evaluated these constructs in cells using GFP reporters harboring corresponding nonsense mutations. Among these, the serine suppressor tRNA demonstrated the most consistent and robust results **(FIG. 16A, FIG. 18A).** To also engineer UAA mediated inducible codon suppression, we next utilized the pyrrolysyl-tRNA/aminoacyl

tRNA synthetase (aaRS) pair from *Methanosarcina barkeri* (MbPylRS)[32,33] and cloned it into AAV vectors. This enabled programmable incorporation of UAAs at a stop codon. Notably, Applicants found that adding a second copy of the tRNA into the expression vector significantly boosted suppression efficiencies **(FIG. 18B).** Applicants further systematically evaluated additional aminoacyl tRNA synthetases from *Methanosarcina mazei* (MmPylRS)[34] and an *N*e-acetyl-lysyl-tRNA synthetase (AcKRS), and also explored varying the number of tRNAs copies per vector to up to four **(FIG. 18B).**

[0278] As suppressor tRNA based approaches can lead to the read-through of other non-target stop codons, concurrently Applicants also engineered a system for sequence-specific targeted RNA editing via adenosine deaminase enzymes. Specifically, adenosine to inosine (A to I) editing is a common post-transcriptional modification in RNA, catalyzed by adenosine deaminases acting on RNA (ADARs). Inosine is a deaminated form of adenosine and is biochemically recognized as guanine. Recently, multiple studies have demonstrated the engineering of ADAR2 mediated targeting *in vitro,* and a study also demonstrated correction of the nonsense mutation in CFTR in xenopus oocytes. Building on this, Applicants engineered here a system for sequence-specific targeted RNA editing *in vitro* and *in vivo,* utilizing the human ADAR2 enzyme and an associated ADAR2 guide RNA (adRNAs) engineered from its naturally occurring substrate GluR2 pre-mRNA. This ADAR2 guiding RNA comprises an ADAR-recruiting domain and a programmable antisense region that is complementary to a specified target RNA sequence. Applicants first evaluated the RNA editing efficiency of this system *in vitro* by co-transfecting the constructs with GFP reporters harboring a non-sense amber or ochre mutation at Y39. Specifically, Applicants utilized two editing approaches to engineer the editing of both adenosines in the ochre stop codon: a one-step mechanism where both the adenosines are edited simultaneously or a two-step mechanism wherein editing takes place sequentially. In addition, we also explored the possibility of conversion of an ochre codon to an amber codon followed by amber suppression to restore GFP expression. All three approaches enabled restoration of GFP expression **(FIG. 16C, FIG. 19A).** Applicants next constructed AAV vectors to deliver the adRNA or a reverse oriented adRNA (radRNA) along with the ADAR2 enzyme. Similar to tRNA mediated codon suppression, addition of a second copy of the adRNA/radRNA

significantly improved the targeting efficiency **(FIG. 19D).** Applicants further systematically evaluated modified ADAR recruiting domains, and a range of RNA targeting antisense designs of varying lengths and the number of nucleotides intervening the target A and the R/G motif of the adRNA[26], yielding a panel of efficient adRNA designs **(FIG. 19B-C).**

[0279] Based on the above *in vitro* optimizations, Applicants next tested the system for *in vivo* RNA targeting. Applicants focused first on the *mdx* mouse model for Duchenne muscular dystrophy (DMD)[35] which bears an ochre stop site in exon 23 of the dystrophin gene. Recent studies utilizing the CRISPR-Cas9 system have shown promising results in the prevention[38] and partial functional recovery of DMD by making changes in exon 23 at the DNA level in the *mdx* mouse. We thus concurrently evaluated three approaches **(FIG. 17A):** one, suppressor tRNAs derived from modified endogenous tRNAs or pyrrolysyl tRNAs for nonsense codon supression; two, ADAR2 based correction of the nonsense mutation; and, three, CRISPR-Cas9 based genome targeting to benchmark the RNA targeting approaches.

[0280] Corresponding, Applicants first designed an AAV carrying two copies of the serine suppressor tRNA targeting the ochre stop codon, and the tibalis anterior (TA) or gastrocnemius of *mdx* mice were injected with the same. Mice muscles were harvested after 2, 4, and 8 weeks. Progressively improved restoration of dystrophin expression was seen over time, with the mice harvested after 8 weeks showing the greatest degree of restoration **(FIG. 17B, FIG. 20A).** In addition, neuronal nitric oxide synthase (nNOS) activity was restored at the sarcolemma which is absent in *mdx* mice due to the absence of the nNOS binding site in the mutant dystrophin protein **(FIG. 17B).** To further make the system inducible, a vector carrying two copies of the pyrrolysyl-tRNA targeting the ochre stop codon and MbPylRS was also constructed and injected into the TA or gastrocnemius of *mdx* mice, and the mice were divided into two groups: one that was administered the pyrrolysine UAA and a control group that was not. Expectedly only mice that were provided the UAA showed nNOS localization at the sarcolemma **(FIG. 20B),** and restoration of dystrophin expression **(FIG. 20C).**

[0281] Next, Applicants evaluated the ADAR2 based site-specific RNA editing approach in this mouse model. To test the efficiency of this system in editing both adenosines in the ochre stop codon in *mdx* DMD mRNA, Applicants first optimized the constructs *in vitro* with a

reporter plasmid bearing a fragment of the *mdx* DMD mRNA in HEK293T cells. Sanger sequencing and NGS analysis confirmed successful targeting **(FIG. 21A).** Applicants next packaged the optimized constructs into AAV8, and injected the tibialis anterior (TA) or gastrocnemius of *mdx* mice. Eight weeks post injection, TA and gastrocnemius muscles were collected from *mdx* mice, wild type mice, and mice treated with adRNA targeting and non-targeting controls. IHC revealed clear restoration of dystrophin expression **(FIG. 17B).** In addition, nNOS activity was also restored at the sarcolemma **(FIG. 17B).** RNA editing rates (TAA->TGG/TAG/TGA) of 0.5-0.7% were observed in treated mice **(FIG. 17C, FIG. 21B).** Applicants also note that the *mdx* mice showed no mRNA with a TAA->TGG change while the treated mice showed up to *0.42%* TAA->TGG edited mRNA. Applicants note that corresponding DNA editing rates via CRISPR-Cas9 in published *in vivo* targeting studies were about 2%[39]. To further benchmark the tRiAD approach, we thus also targeted the *mdx* mice via CRISPR based genome editing of the nonsense mutation. Applicants injected vectors bearing dual-gRNAs to excise exon 23 codon, and expectedly, this led to restoration of dystrophin expression in a subset of the muscle cells **(FIG. 17B).**

[0282] Finally, we also evaluated the ADAR2 mediated RNA editing approach in an independent mouse model of human disease. Specifically, we focused on the male sparse fur ash *(spf^{ash})* mouse model of ornithine transcarbamylase (OTC) deficiency. The *spf^{sh}* mice have a G->A point mutation in the last nucleotide of the fourth exon of the OTC gene, which leads to OTC mRNA deficiency and production of mutant protein[43]. Recent studies have demonstrated the use of CRISPR-Cas9 and homologous recombination based strategies for robust correction of this mutation in neonatal mice. However, gene correction via homology-directed repair (HDR) in adult mice was inefficient and led to genomic deletions which proved to be lethal as they compromised liver function in targeted mice. To test the effectiveness of the system in editing the point mutation in *spf^{sh}* OTC mRNA **(FIG. 17D),** Applicants first evaluated our constructs *in vitro* with a plasmid bearing a fragment of the *spf^{sh}* OTC mRNA in HEK293T cells. Sanger sequencing and next generation sequencing (NGS) analysis confirmed robust RNA editing efficiencies **(FIG. 21C).** Applicants next packaged the constructs into AAV8, which has high liver tropism[44], and injected 10-12 week old *spf^{sh}* mice. Four weeks post injection, Applicants collected liver samples from *spf^{ash}*,

wild-type litter mates, and *spf* ^{sh} mice treated with the ADAR2 targeting and non-targeting vectors and evaluated editing efficiency via NGS. Notably, significant RNA editing rates in the range of 0.8-4.2% were observed in treated mice in the spliced OTC mRNA **(FIG. 17E, FIG. 21D),** further confirming the utility of this approach for *in vivo* editing of endogenous RNA in adult mice.

[0283] Taken together, Applicants' results establish the use of suppressor tRNAs and ADAR2 as potential strategies for *in vivo* RNA targeting of point mutations. Specifically, by optimizing delivery, Applicants first demonstrated robust and inducible stop codon read-through via the use of suppressor tRNAs. The delivery of modified endogenous suppressor tRNAs for premature stop read-through has several potential advantages: it lacks the toxicity associated with read-through drugs such as gentamycin and can be used to bring about efficient stop codon read-through in post-mitotic cells. In addition, being of endogenous origin, it is not likely to elicit a strong immune response. Additionally, the inducibility enabled by the UAA based systems, albeit non-native, could provide tight regulation over the expression of genes. Localized injections of the UAA into the target muscle could further help improve the efficiency of the system in future studies. Notably, Applicants did not observe any overt toxicity via this approach in the *mdx* targeting studies. Applicants however note too that an important caveat to this strategy, analogous to the read-through drugs, is that suppressor tRNA based approaches will lead to the read-through of other non-target stop codons. In this regard, Applicants thus also demonstrated ADAR2 based site-specific correction of point mutations in RNA in two independent mouse models. Applicants note that potential off-targets in RNA are limited as compared to DNA, as the transcriptome is only a small subset of the genome. Secondly, even if off-targets exist, the presence of an A within the target window is required for the enzyme to create an off-target A->G change. Lastly, the off-target effects will be transient. Thus, overall off-target effects due to a RNA editing enzyme such as ADAR2 are likely to be limited, although enzyme processivity, promiscuity, and off-target hybridization of the antisense domain of the adRNA need to be studied thoroughly. ADAR2 being of human origin is also less likely to elicit an immune response, while enabling more site-specific editing of RNA compared to the suppressor tRNA approach.

[0284] Applicants also note that compared to the tRiAD based RNA targeting approaches above, CRISPR based genome targeting approaches currently show faster kinetics and greater degree of mutant protein restoration. Applicants however anticipate that systematic engineering and directed evolution of the ADAR2 could help improve the editing efficiency and also eliminate the intrinsic biases of the ADAR2 for certain sequences, coupled with insights from studies unearthing novel regulators of ADAR2 providing cues to improve its stability. In this regard, Applicants tested the ADAR2-E488Q mutant and noted that it enabled higher editing efficiency than the wild type ADAR2 for both the DMD and OTC mRNA fragments expressed *in vitro* **(FIG. 22).** The demonstration of site-specific A->G mRNA editing *in vivo* also opens up the door to future site-specific C->T editing via targeted recruitment of cytosine deaminases, thereby potentially expanding the repertoire of RNA editing tools. However, an important consideration while targeting RNA for gene therapy via the use of non-integrating vectors such as AAVs, is the necessity for periodic re-administration of the effector constructs due to the typically limited half-life of edited mRNAs. Secondly, RNA folding, intrinsic half-life, localization, and RNA binding proteins might also impact accessibility of target sites in the RNA. For instance, in this example, the short half-life of mutant dystrophin RNA, and the need to target the transient pre-mRNA in OTC potentially negatively impact overall editing efficiencies. Chemical and structural modifications in tRNAs and adRNAs while taking cues from the specificity studies on sgRNAs[49], or coupling of shielding proteins, or recently demonstrated programmable RNA binding proteins and RNA-targeting CRISPR-Cas systems, might help improve RNA stability and specificity, and improve the efficiency of the above approaches. With progressive improvements, Applicants thus anticipate this integrated tRiAD toolset will have broad implications in both applied life sciences as well as fundamental research.

Example 6 - ADAR and APOBEC editing efficacy

[0285] A number of ADAR scaffolds - both dual and single - were tested for efficacy in recruiting ADAR in a cell line where ADAR2 was overexpressed **(FIG. 28** and **FIG. 29).** Further assessments were made for MCP-ADAR fusion scaffolds **(FIG. 30).** Endogenous mRNA target editing efficiency was assessed using scaffold v2.

mRNA	Target	#1	#2	#3	Average
RAB7A	GGGAAATCTAGCTAGCGGCA	32.0%	34.1%	30.2%	32.1%
RAB7A	GGGAAAACTGTCTAGTTCTC	28.2%	27.5%	23.0%	26.2%
CCNB1	TAATTGACTGGCTAGTACAG	23.8%	17.2%	21.1%	20.7%
CCNB1	GAGCTTTTTGCTTAGCACTG	15.1%	18.4%	17.4%	17.0%

Equivalents

[0286] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs.

[0287] The present technology illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising," "including," "containing," *etc.* shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the present technology claimed.

[0288] Thus, it should be understood that the materials, methods, and examples provided here are representative of preferred aspects, are exemplary, and are not intended as limitations on the scope of the present technology.

[0289] The present technology has been described broadly and generically herein. Each of the narrower species and sub-generic groupings falling within the generic disclosure also form part of the present technology. This includes the generic description of the present technology with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0290] In addition, where features or aspects of the present technology are described in terms of Markush groups, those skilled in the art will recognize that the present technology is

also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0291] All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety, to the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control.

[0292] Other aspects are set forth within the following claims.

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WHAT IS CLAIMED IS:

1. A method for restoring expression of a protein comprising a point mutation in an RNA sequence encoding the protein in a subject in need thereof comprising administering to the subject a vector encoding one or more tRNA having an anticodon sequence that recognizes a codon comprising the point mutation, optionally wherein the point mutation results in a premature stop codon.

2. The method of claim 1, wherein the point mutation results in a nonsense mutation having the DNA sequence TAA and the RNA sequence UAA.

3. The method of claim 1 or 2, wherein the tRNA is an endogenous tRNA with a modified anticodon stem recognizing the codon comprising the point mutation.

4. The method of claim 3, wherein the tRNA is charged with a serine.

5. The method of claim 1 or 2, wherein the tRNA is an orthogonal tRNA charged with a non-canonical amino acid.

6. The method of claim 5, wherein the vector further comprises a corresponding tRNA synthetase.

7. The method of claim 5 or 6, wherein the non-canonical amino acid is pyrrolysine.

8. The method of claim 7, wherein the method further comprises introducing pyrrolysine in diet of the subject.

9. The method of any one of the preceding claims, wherein the vector encodes two tRNA having an anticodon sequence that recognizes the codon comprising the point mutation.

10. The method of any one of the preceding claims, wherein the gene is dystrophin.

11. A method of treating a disease, disorder, or condition characterized by the presence of a point mutation in an RNA sequence encoding a gene associated with the disease, disorder,

or condition in a subject in need thereof comprising administering to the subject a vector encoding one or more tRNA having an anticodon sequence that recognizes a codon comprising the point mutation, optionally wherein the point mutation results in a premature stop codon.

12. The method of any one of claims 11, wherein the point mutation results in a nonsense mutation having the DNA sequence TAA and the RNA sequence UAA.

13. The method of claim 11 or 12, wherein the tRNA is an endogenous tRNA with a modified anticodon stem recognizing the codon comprising the point mutation.

14. The method of claim 13, wherein the tRNA is charged with a serine.

15. The method of claim 11 or 12, wherein the tRNA is an orthogonal tRNA charged with a non-canonical amino acid.

16. The method of claim 15, wherein the vector further comprises a corresponding tRNA synthetase.

17. The method of claim 15 or 16, wherein the non-canonical amino acid is pyrrolysine.

18. The method of claim 17, wherein the method further comprises introducing pyrrolysine in diet of the subject.

19. The method of any one of claims 11 to 18, wherein the vector encodes two tRNA having an anticodon sequence that recognizes codon comprising the point mutation.

20. The method of any one of claims claim 11 to 19, wherein the disease, disorder, or condition is selected from the group consisting of the diseases, disorders, and conditions listed in Table 1, optionally characterized by the presence of a nonsense mutation and/or a premature stop codon.

21. The method of any one of claims 11 to 20, wherein the gene is dystrophin.

22. The method of claim 21, wherein the disease, disorder, or condition is muscular dystrophy.

23. The method of claim 22, wherein the muscular dystrophy is Duchene muscular dystrophy.

24. A vector encoding one or more tRNA having an anticodon sequence that recognizes a codon comprising a point mutation in an RNA sequence encoding a gene, optionally wherein the point mutation results in a premature stop codon.

25. The vector of claim 24, wherein the point mutation results in a nonsense mutation having the DNA sequence TAA and the RNA sequence UAA.

26. The vector of claim 24 or 25, wherein the tRNA is an endogenous tRNA with a modified anticodon stem recognizing the codon comprising the point mutation.

27. The vector of claim 26, wherein the tRNA is charged with a serine.

28. The vector of claim 24 or 25, wherein the tRNA is an orthogonal tRNA charged with a non-canonical amino acid.

29. The vector of claim 28, wherein the vector further comprises a corresponding tRNA synthetase.

30. The vector of claim 28 or 29, wherein the non-canonical amino acid is pyrrolysine.

31. The vector of any one of claims 24-30, wherein the vector is an AAV vector.

32. The vector of claim 31, wherein the vector is an AAV8 vector.

33. The vector of any one of claims 24-32, wherein the vector encodes two tRNA having an anticodon sequence that recognizes the codon comprising the point mutation.

34. The vector of any one of claims 24-33, wherein the gene is dystrophin.

35. A method for restoring expression of a protein comprising a point mutation in an RNA sequence encoding the protein in a subject in need thereof comprising administering one or more vectors encoding an ADAR based RNA editing system comprising one or more forward guide RNAs for the ADAR ("adRNAs") and one or more corresponding reverse guide RNAs for the ADAR ("radRNAs") to the subject, wherein the ADAR based RNA editing system specifically edits the point mutation.

36. The method of claim 35, wherein the point mutation results in a nonsense mutation having the DNA sequence TAA and the RNA sequence UAA.

37. The method of claim 36, wherein the ADAR based RNA editing system converts UAA to UIA.

38. The method of claim 37, wherein the ADAR based RNA editing system converts UIA to UII.

39. The method of claim 36, wherein the ADAR based RNA editing system converts UAA to UAL

40. The method of claim 39, wherein the one or more vector further encodes a tRNA that targets an amber codon.

41. The method of any one of claims 35 to 40, wherein the protein is dystrophin.

42. The method of claim 35, wherein the point mutation results in a splice site or missense mutation having the DNA sequence CAG and the RNA sequence CAG.

43. The method of claim 42, wherein the ADAR based RNA editing system converts CAG to CIG.

44. The method of any one of claims 35, 42, or 43, wherein the protein is ornithine transcabamylase.

45. The method of any one of claims 35 to 44, wherein the ADAR based editing system further comprises ADARl, ADAR2, the E488Q and E1008Q mutants each thereof, a fusion

protein comprising the catalytic domain of an ADAR and a domain which associates with an RNA hairpin motif, a fusion protein comprising the catalytic domain of an ADAR and a dead Cas9, or a fusion protein comprising the double stranded binding domain of an ADAR and an APOBEC.

46. The method of claim 45, wherein the domain which associates with an RNA hairpin motif is selected from the group of an MS2 bacteriophage coat protein (MCP) and an N22 peptide.

47. The method of any one of claims 35 to 46, wherein the method further comprises administering an effective amount of an interferon to enhance endogenous ADARl expression.

48. The method of claim 47, wherein the interferon is interferon a.

49. The method of any one of claims 35 to 48, wherein the adRNA comprises one or more RNA hairpin motifs.

50. The method of claim 49, wherein the one or more RNA hairpin motifs are selected from the group of an MS2 stem loop and a BoxB loop.

51. The method of claim 49, wherein the one or more RNA hairpin motifs are stabilized by replacing A-U with G-C.

52. The method of any one of claims 35 to 51, wherein the adRNA is stabilized through the incorporation of one or more of 2'-0-methyl, 2'-0-methyl 3'phosphorothioate, or 2'-0-methyl 3'thioPACE at either or both termini of the adRNA.

53. A method of treating a disease, disorder, or condition by the presence of a point mutation in an RNA sequence encoding a protein associated with the disease, disorder, or condition in a subject in need thereof comprising administering one or more vectors encoding an ADAR based RNA editing system comprising one or more forward guide RNAs for the ADAR ("adRNAs") and one or more corresponding reverse guide RNAs for the ADAR

("radRNAs") to the subject, wherein the ADAR based RNA editing system specifically edits the point mutation.

54. The method of claim 53, wherein the point mutation results in a nonsense mutation having the DNA sequence TAA and the RNA sequence UAA.

55. The method of claim 54, wherein the ADAR based RNA editing system converts UAA to UIA.

56. The method of claim 53, wherein the ADAR based RNA editing system converts UIA to UII.

57. The method of claim 54, wherein the ADAR based RNA editing system converts UAA to UAL

58. The method of claim 57, wherein the one or more vector further encodes a tRNA that targets an amber codon.

59. The method of any one of claims 53 to 58, wherein the protein is dystrophin.

60. The method of claim 53, wherein the point mutation results in a splice site or missense mutation having the DNA sequence CAG and the RNA sequence CAG.

61. The method of claim 60, wherein the ADAR based RNA editing system converts CAG to CIG.

62. The method of any one of claims 53, 60, or 61, wherein the protein is ornithine transcabamylase.

63. The method of any one of claims 53 to 62, wherein the ADAR based editing system further comprises ADARl, ADAR2, the E488Q and E1008Q mutants each thereof, a fusion protein comprising the catalytic domain of an ADAR and a domain which associates with an RNA hairpin motif, a fusion protein comprising the catalytic domain of an ADAR and a dead Cas9, or a fusion protein comprising the double stranded binding domain of an ADAR and an APOBEC.

64. The method of claim 63, wherein the domain which associates with an RNA hairpin motif is selected from the group of an MS2 bacteriophage coat protein (MCP) and an N22 peptide.

65. The method of any one of claims 53 to 64, wherein the method further comprises administering an effective amount of an interferon to enhance endogenous ADAR1 expression.

66. The method of claim 65, wherein the interferon is interferon a.

67. The method of any one of claims 53 to 66, wherein the adRNA comprises one or more RNA hairpin motifs.

68. The method of claim 67, wherein the one or more RNA hairpin motifs are selected from the group of an MS2 stem loop and a BoxB loop.

69. The method of claim 68, wherein the one or more RNA hairpin motifs are stabilized by replacing A-U with G-C.

70. The method of any one of claims 50 to 69, wherein the adRNA is stabilized through the incorporation of one or more of 2'-0-methyl, 2'-0-methyl 3'phosphorothioate, or 2'-0-methyl 3'thioPACE at either or both termini of the adRNA.

71. The method of any one of claims claim 53 to 70, wherein the disease, disorder, or condition is selected from the group consisting of the diseases, disorders, and conditions listed in Table 1.

72. The method of claim 71, wherein the protein is dystrophin and the disease, disorder, or condition is muscular dystrophy.

73. The method of claim 72, wherein the muscular dystrophy is Duchene muscular dystrophy.

74. A recombinant expression system comprising one or more vectors encoding an ADAR based RNA editing system comprising one or more forward guide RNAs for the

ADAR ("adRNAs") and one or more corresponding reverse guide RNAs for the ADAR ("radRNAs") to the subject, wherein the ADAR based RNA editing system specifically edits a point mutation in an RNA sequence encoding a protein.

75. The recombinant expression system of claim 74, wherein the point mutation results in a nonsense mutation having the DNA sequence TAA and the RNA sequence UAA.

76. The recombinant expression system of claim 75, wherein the ADAR based RNA editing system converts UAA to UIA.

77. The recombinant expression system of claim 76, wherein the ADAR2 based RNA editing system converts UIA to UII.

78. The recombinant expression system of claim 75, wherein the ADAR2 based RNA editing system converts UAA to UAL

79. The recombinant expression system of claim 78, wherein the one or more vector further encodes a tRNA that targets an amber codon.

80. The recombinant expression system of claims 74 to 79, wherein the protein is dystrophin.

81. The recombinant expression system of claim 74, wherein the point mutation results in a splice site or missense mutation having the DNA sequence CAG and the RNA sequence CAG.

82. The recombinant expression system of claim 81, wherein the ADAR based RNA editing system converts CAG to CIG.

83. The recombinant expression system of any one of claims 74, 81, or 82, wherein the protein is ornithine transcabamylase.

84. The recombinant expression system of any one of claims 74 to 83, wherein the ADAR based editing system further comprises ADARl, ADAR2, the E488Q and E1008Q mutants each thereof, a fusion protein comprising the catalytic domain of an ADAR and a domain

which associates with an RNA hairpin motif, a fusion protein comprising the catalytic domain of an ADAR and a dead Cas9, or a fusion protein comprising the double stranded binding domain of an ADAR and an APOBEC.

85. The recombinant expression system of claim 84, wherein the domain which associates with an RNA hairpin motif is selected from the group of an MS2 bacteriophage coat protein (MCP) and an N22 peptide.

86. The recombinant expression system of any one of claims 74 to 85, wherein the adRNA comprises one or more RNA hairpin motifs.

87. The recombinant expression system of claim 86, wherein the one or more RNA hairpin motifs are selected from the group of an MS2 stem loop and a BoxB loop.

88. The recombinant expression system of claim 86, wherein the one or more RNA hairpin motifs are stabilized by replacing A-U with G-C.

89. The recombinant expression system of any one of claims 74 to 88, wherein the adRNA is stabilized through the incorporation of one or more of 2'-0-methyl, 2'-0-methyl 3'phosphorothioate, or 2'-0-methyl 3'thioPACE at either or both termini of the adRNA.

90. The recombinant expression system of any one of claims 74 to 89, wherein the protein is dystrophin or ornithine transcabamylase.

91. A composition comprising the recombinant expression system of any one of claims 74 to 90, and an effective amount of an interferon to enhance endogenous ADARl expression

92. The composition of claim 91, wherein the interferon is interferon a.

FIGURE 1

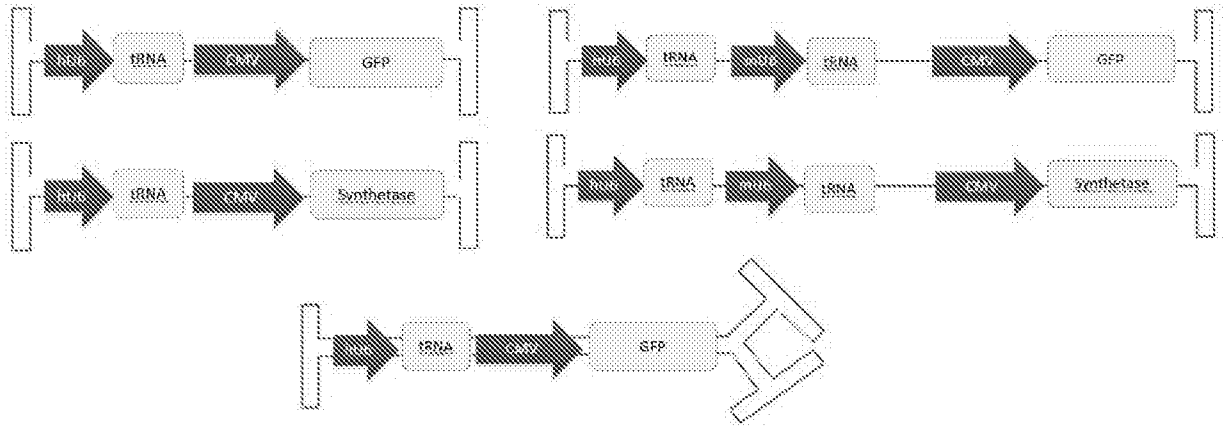

tRNA
GFP
tRNA
Synthetase
tRNA
tRNA
GFP
tRNA
tRNA
Synthetase
tRNA
GFP

FIGURE 2

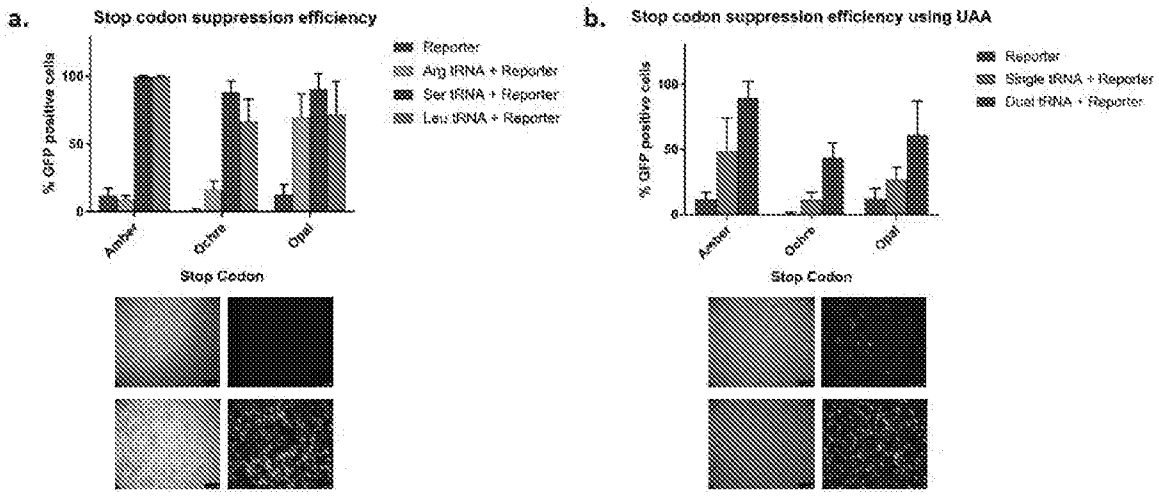

a.
Stop codon suppression efficiency
% GFP positive cells
100
50
0
Amber
Ochre
Opal
Stop Codon
Reporter
Arg tRNA + Reporter
Ser tRNA + Reporter
Leu tRNA + Reporter
b.
Stop codon suppression efficiency using UAA
% GFP positive cells
100
50
0
Amber
Ochre
Opal
Stop Codon
Reporter
Single tRNA + Reporter
Duel tRNA + Reporter

FIGURE 3

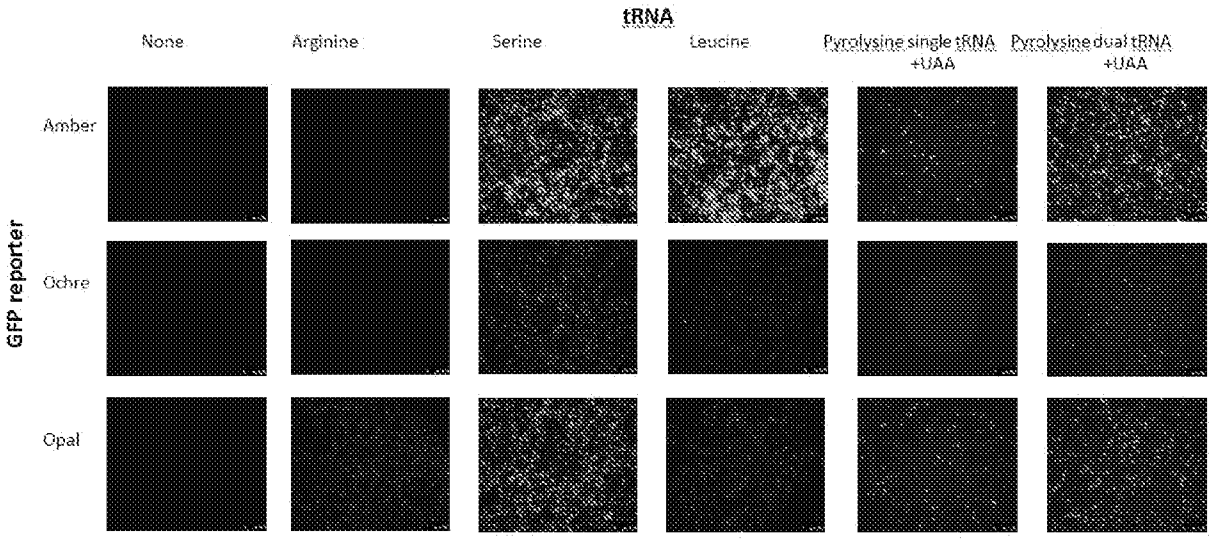

tRNA
None
Arginine
Serine
Leucine
Pyrolysine single tRNA +UAA
Pyrolysine dual tRNA +UAA
GFP reporter
Amber
Ochre
Opal

FIGURE 4

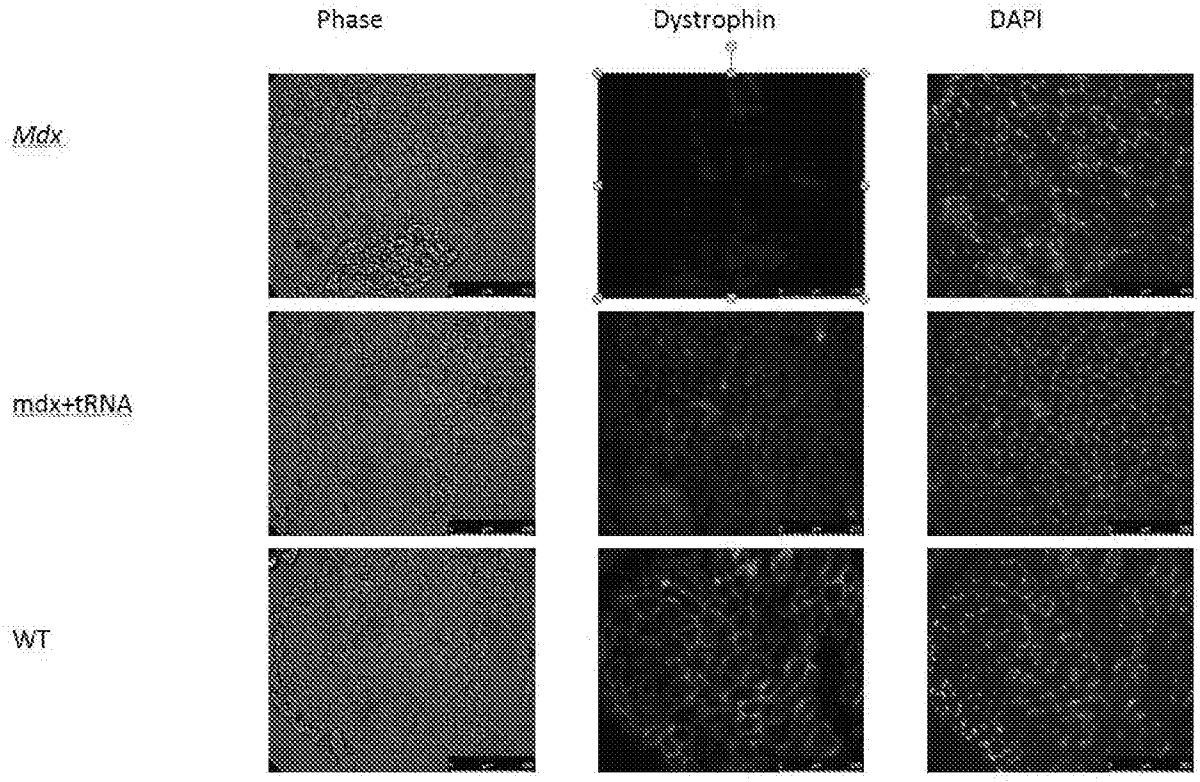
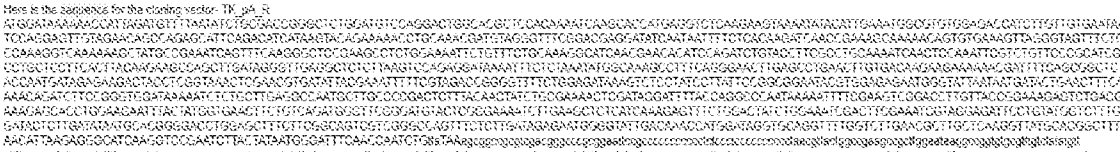
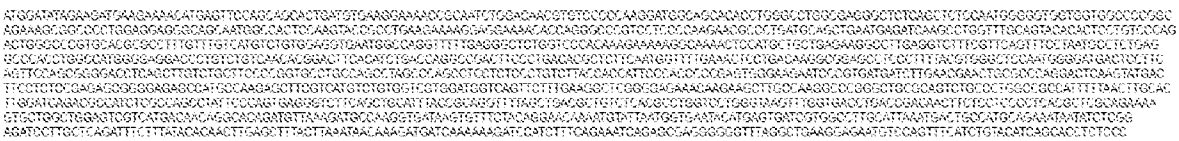


FIGURE 5

Here is the sequence for the cloning vector- TK_pA_R



For the ADAR2 constructs - TK_pA_R:



ADAR2 constructs - pUK01_5

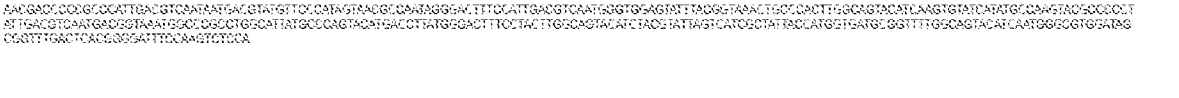


FIGURE 6

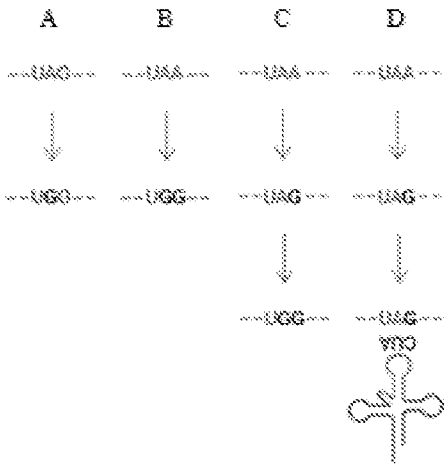


FIGURE 7

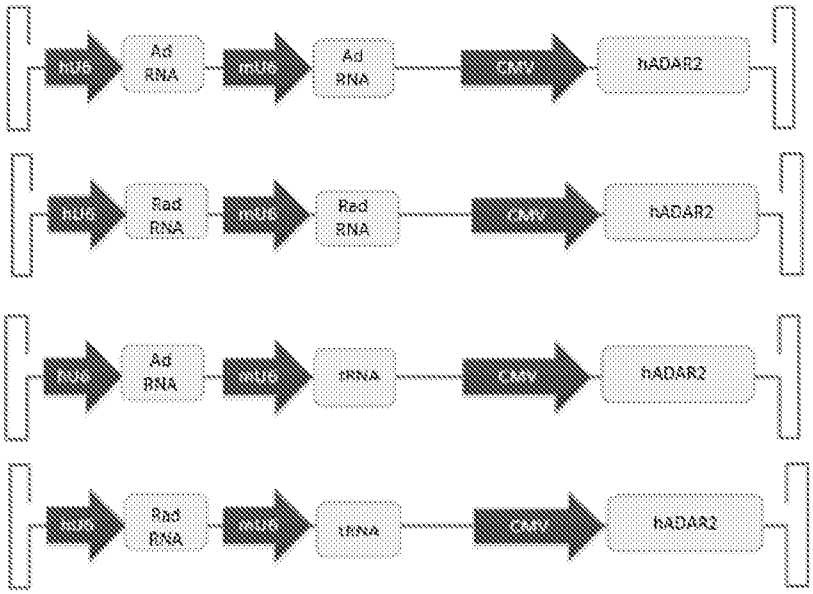

Ad RNA
Ad RNA
hADAR2
Rad RNA
Rad RNA
hADAR2
Ad RNA
hADAR2
Rad RNA
hADAR2

FIGURE 8

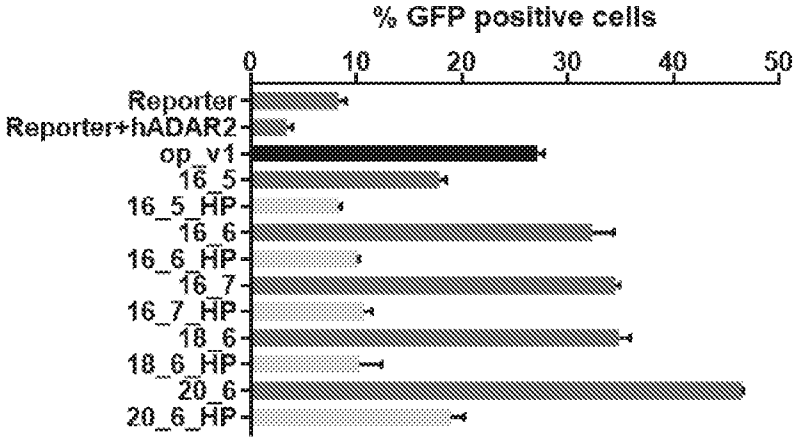

% GFP positive cells
0
10
20
30
40
50
Reporter
Reporter+hADAR2
op_v1
16_5
16_5_HP
16_6
16_6_HP
16_7
16_7_HP
18_6
18_6_HP
20_6
20_6_HP

FIGURE 9

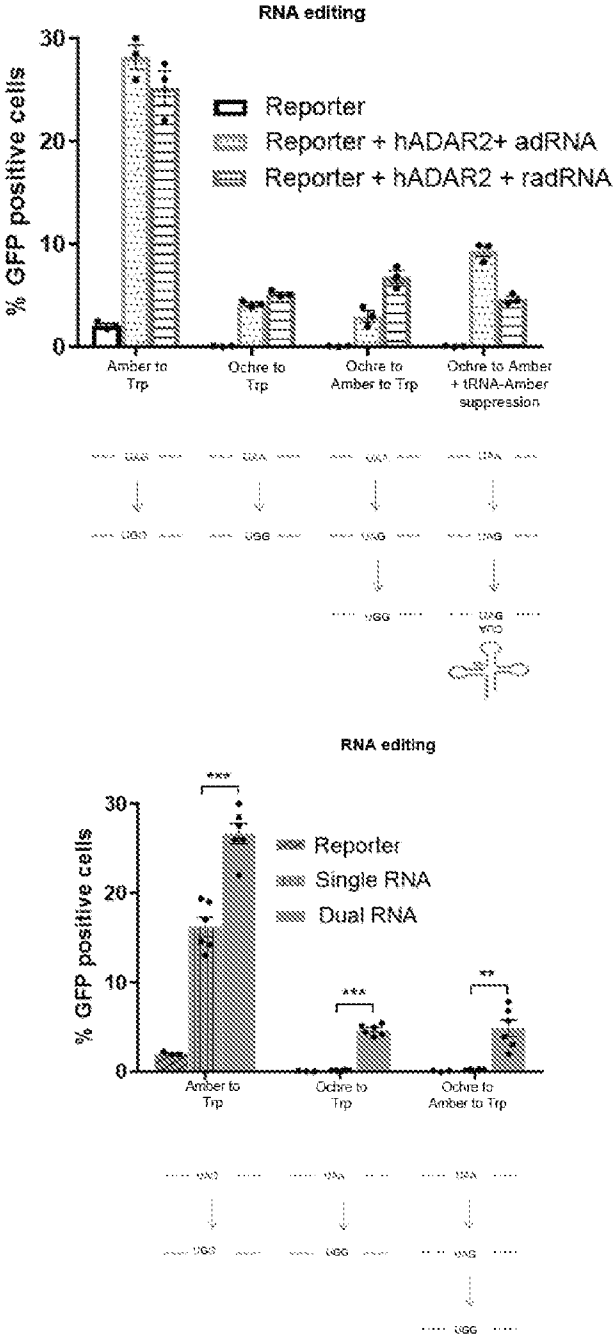
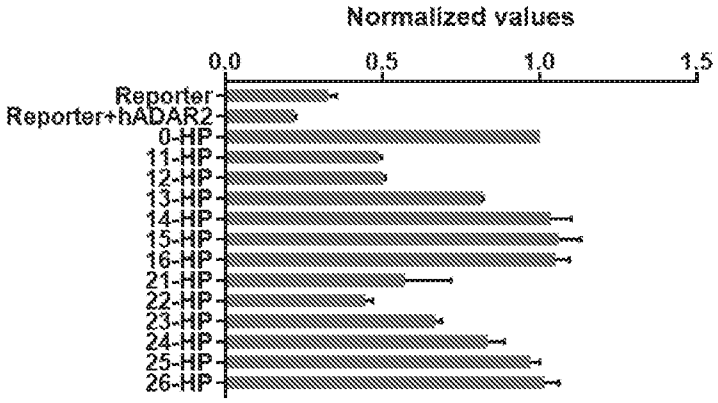

RNA editing
% GFP positive cells
30
20
10
0
Reporter
Reporter + hADAR2+ adRNA
Reporter + hADAR2 + radRNA
Amber to Trp
Ochre to Trp
Ochre to Amber to Trp
Ochre to Amber + tRNA-Amber suppression
RNA editing
% GFP positive cells
30
20
10
0
***
***
**
Reporter
Single RNA
Dual RNA
Amber to Trp
Ochre to Trp
Ochre to Amber to Trp

FIGURE 10



agggcgatgccacctGGgg
agggcgatgcgacctgGgg
agggcgatgggacctgGgg
atcgccctgaaaagggcgatgccacctGGgg
atcgccctgaaaagggcgatgcgacctgGgg
atcgccctgaaaagggcgatgggacctgGgg

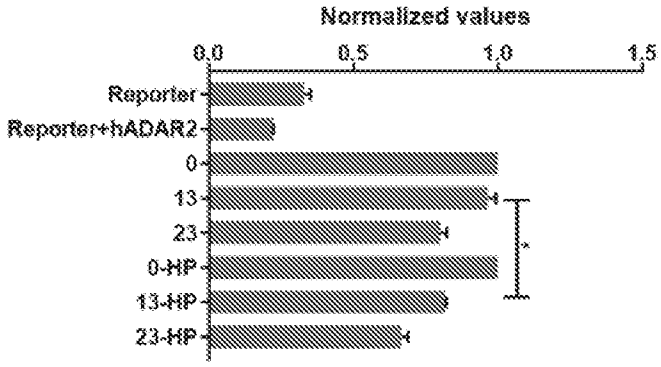


FIGURE 11

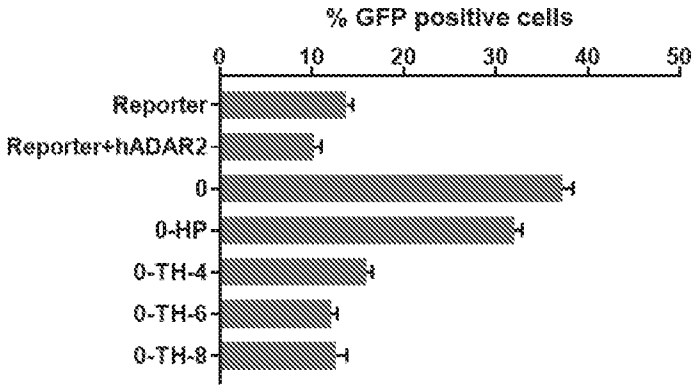
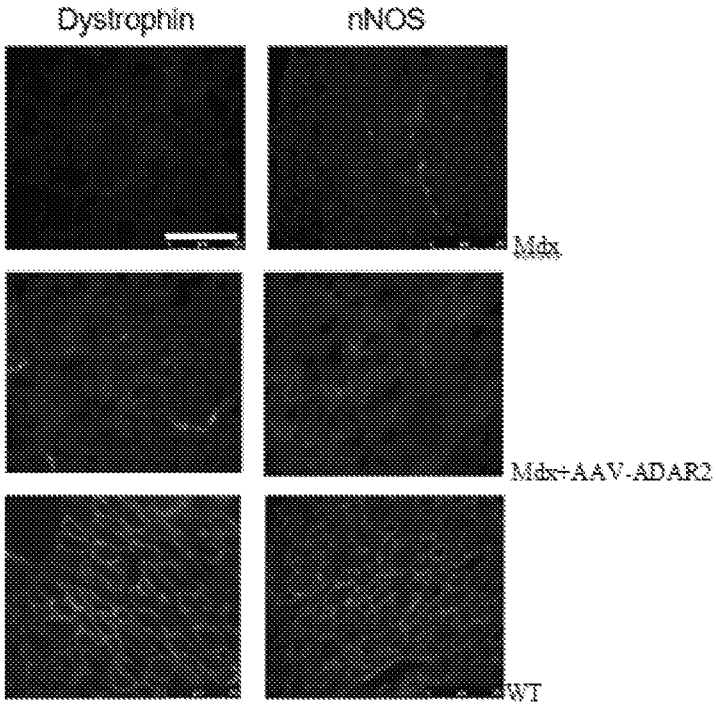
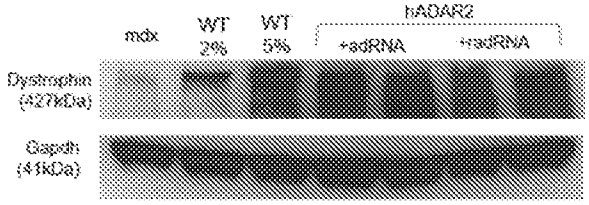

% GFP positive cells
0
10
20
30
40
50
Reporter
Reporter+hADAR2
0
0-HP
0-TH-4
0-TH-6
0-TH-8

FIGURE 12

(a)



(b)



(c)

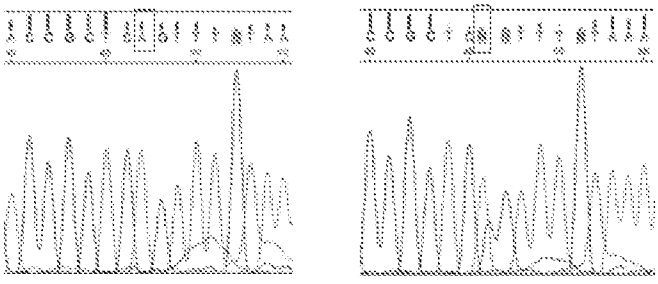


FIGURE 13

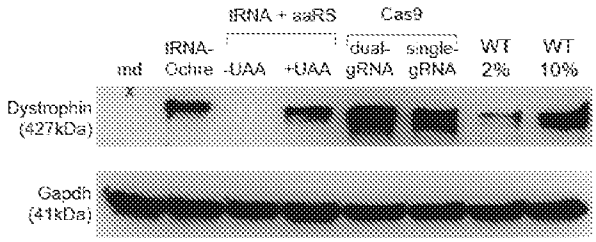

tRNA + saRS
Cas9
mdx
tRNA-Ochre
-UAA
+UAA
dual-gRNA
single-gRNA
WT 2%
WT 10%
Dystrophin (427kDa)
Gapdh (41kDa)

FIGURE 14

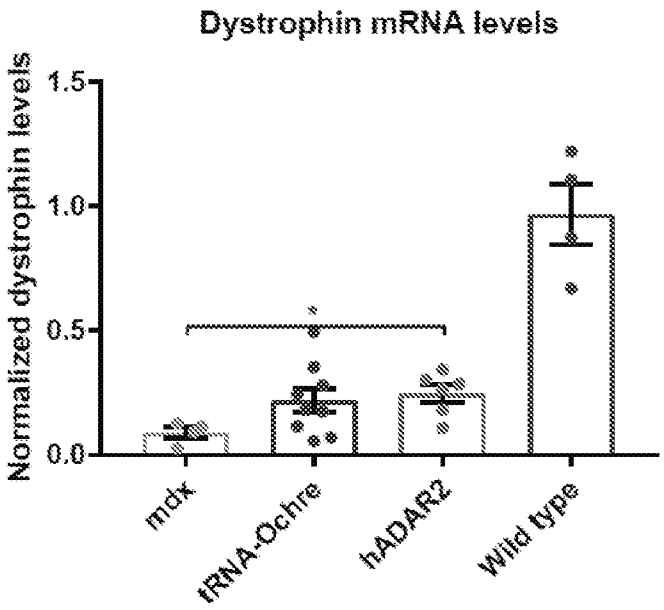

Dystrophin mRNA levels
Normalized dystrophin levels
1.5
1.0
0.5
0.0
mdx
tRNA-Ochre
hADAR2
Wild type

FIGURE 15

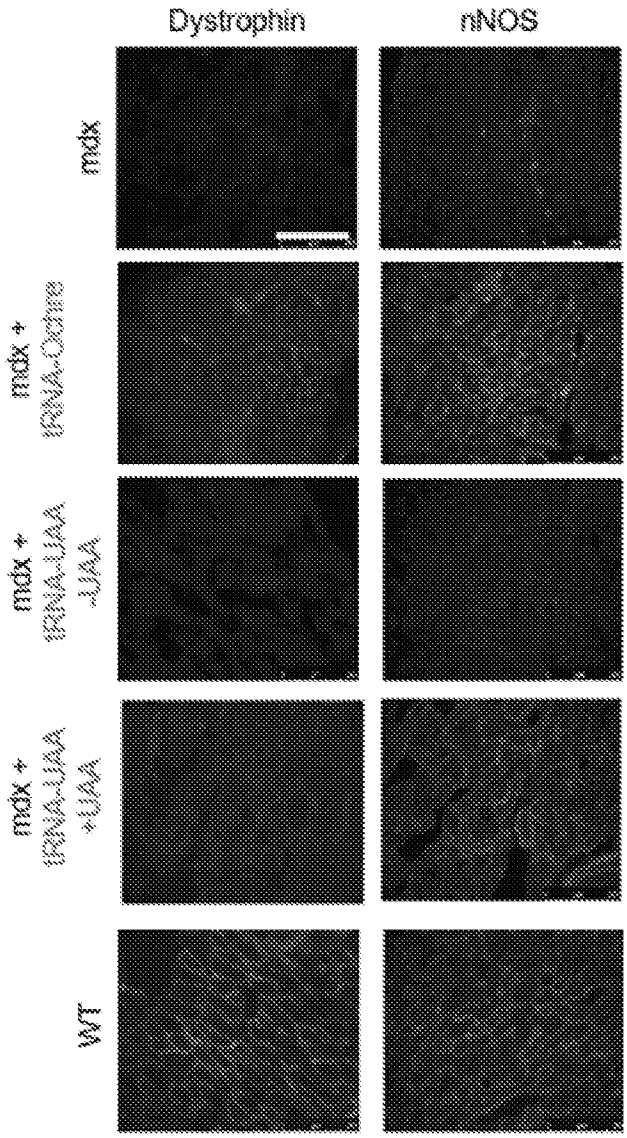

Dystrophin
nNOS
mdx
mdx + tRNA-Ochre
mdx + tRNA-UAA -UAA
mdx + tRNA-UAA +UAA
WT

FIGURE 16

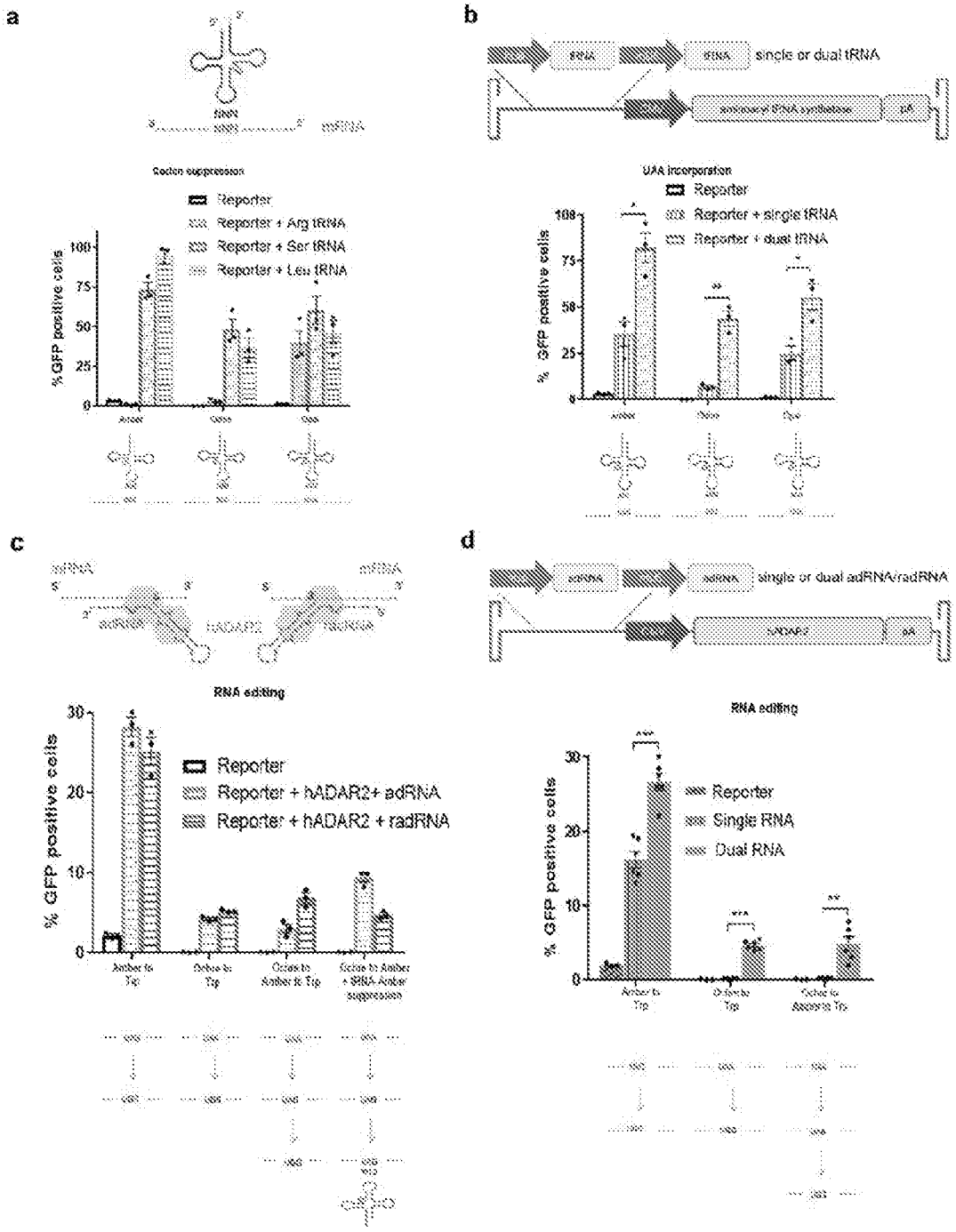

a
b
c
d
Codon suppression
Reporter
Reporter + Arg tRNA
Reporter + Ser tRNA
Reporter + Leu tRNA
% GFP positive cells
UAA incorporation
Reporter
Reporter + single tRNA
Reporter + dual tRNA
single or dual tRNA
RNA editing
Reporter
Reporter + hADAR2+ adRNA
Reporter + hADAR2 + radRNA
Reporter
Single RNA
Dual RNA
single or dual adRNA/radRNA

FIGURE 17

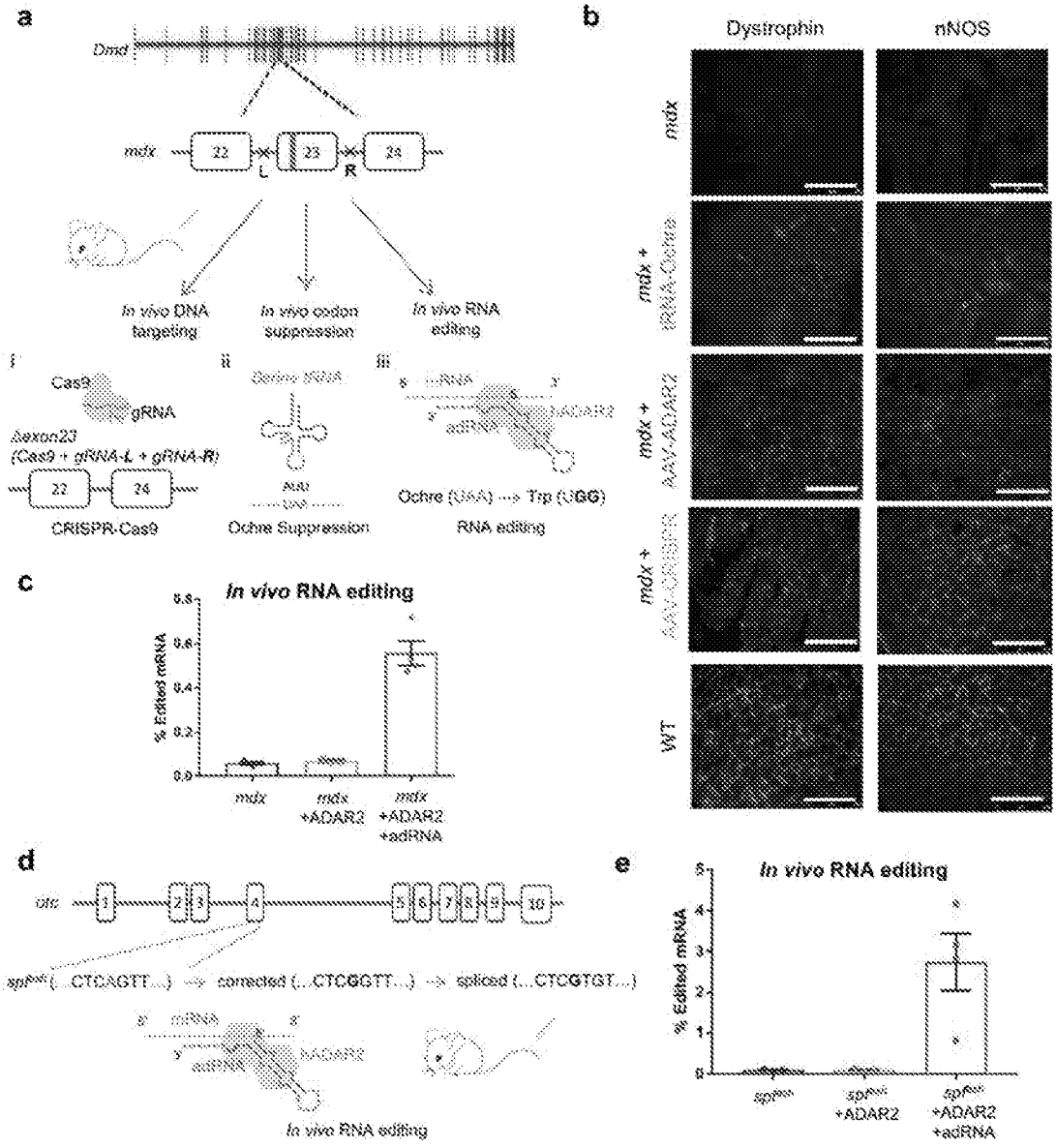

a
Dmd
mdx
22
23
24
L
R
In vivo DNA targeting
In vivo codon suppression
In vivo RNA editing
i
Cas9
gRNA
Δexon23
(Cas9 + gRNA-L + gRNA-R)
22
24
CRISPR-Cas9
ii
Donor RNA
AUG
UAA
Ochre Suppression
iii
ADAR2
adRNA
Ochre (UAA) → Trp (UGG)
RNA editing
b
Dystrophin
nNOS
mdx
mdx + tRNA-Ochre
mdx + AAV-ADAR2
mdx + AAV-ADAR2-tRNA
WT
c
In vivo RNA editing
% Edited mRNA
0.0
0.2
0.4
0.6
0.8
mdx
mdx +ADAR2
mdx +ADAR2 +adRNA
d
otc
1
2
3
4
5
6
7
8
9
10
spf^ash (...CTCAGTT...) → corrected (...CTCGGTT...) → spliced (...CTCGTGT...)
In vivo RNA editing
e
In vivo RNA editing
% Edited mRNA
0
1
2
3
4
5
spf^ash
spf^ash +ADAR2
spf^ash +ADAR2 +adRNA

FIGURE 18

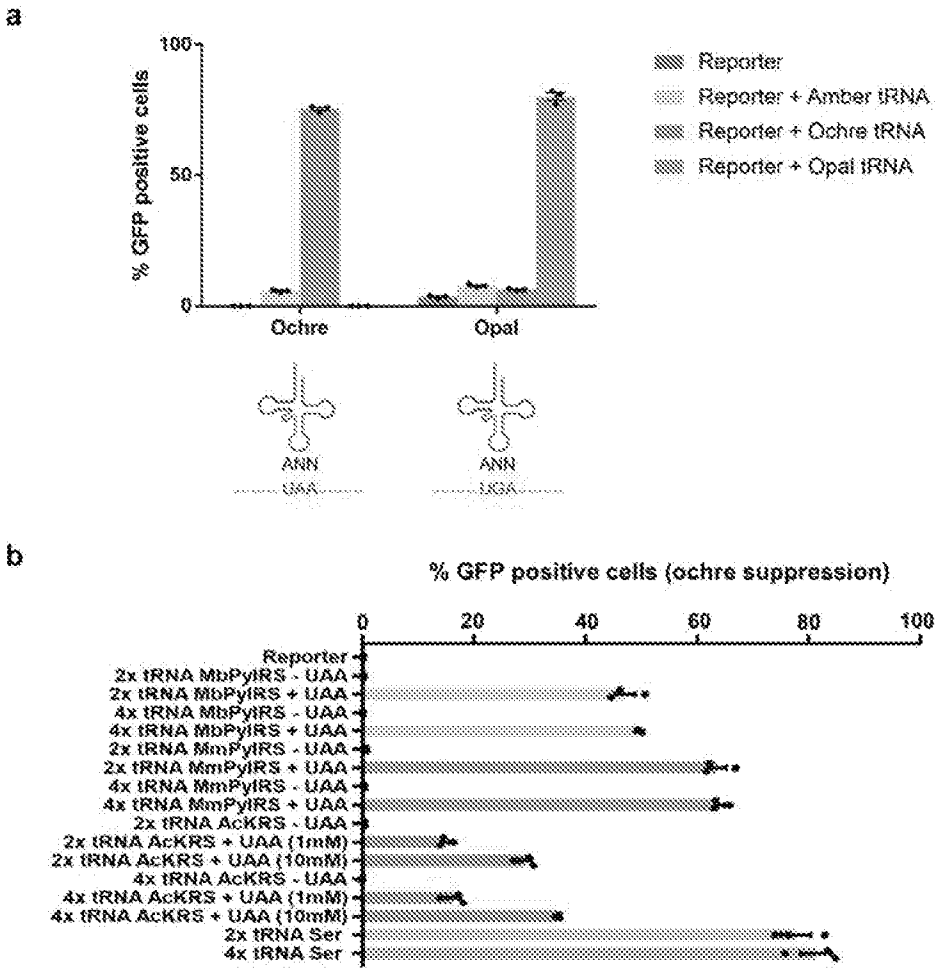

a
% GFP positive cells
100
50
0
Ochre
Opal
ANN
UAA
ANN
UGA
Reporter
Reporter + Amber tRNA
Reporter + Ochre tRNA
Reporter + Opal tRNA
b
% GFP positive cells (ochre suppression)
0
20
40
60
80
100
Reporter
2x tRNA MbPylRS - UAA
2x tRNA MbPylRS + UAA
4x tRNA MbPylRS - UAA
4x tRNA MbPylRS + UAA
2x tRNA MmPylRS - UAA
2x tRNA MmPylRS + UAA
4x tRNA MmPylRS - UAA
4x tRNA MmPylRS + UAA
2x tRNA AcKRS - UAA
2x tRNA AcKRS + UAA (1mM)
2x tRNA AcKRS + UAA (10mM)
4x tRNA AcKRS - UAA
4x tRNA AcKRS + UAA (1mM)
4x tRNA AcKRS + UAA (10mM)
2x tRNA Ser
4x tRNA Ser

FIGURE 19

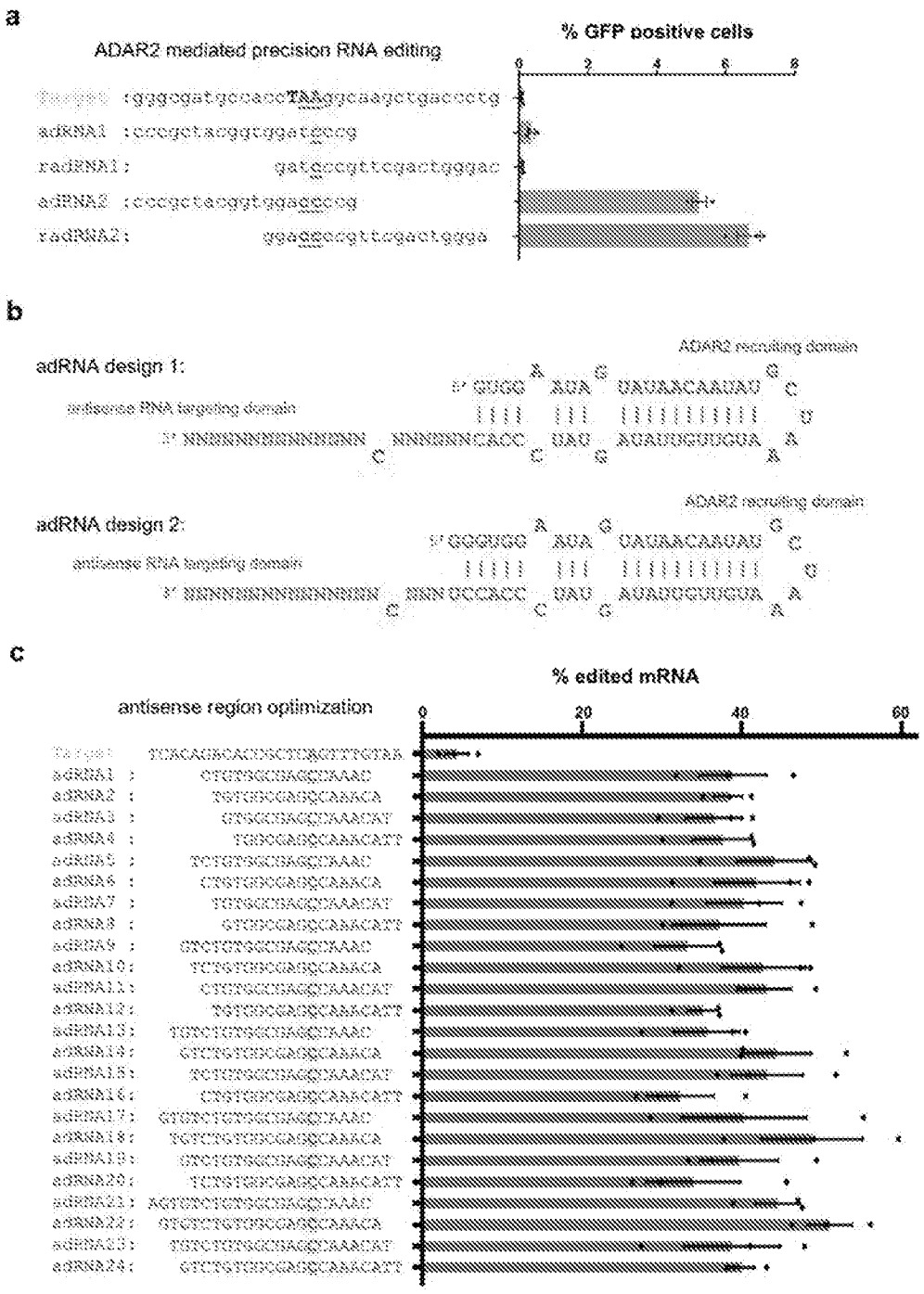

a
ADAR2 mediated precision RNA editing
% GFP positive cells
0
2
4
6
8
adRNA1
radRNA1
adRNA2
radRNA2
b
adRNA design 1:
ADAR2 recruiting domain
antisense RNA targeting domain
adRNA design 2:
ADAR2 recruiting domain
antisense RNA targeting domain
c
antisense region optimization
% edited mRNA
0
20
40
60
adRNA1
adRNA2
adRNA3
adRNA4
adRNA5
adRNA6
adRNA7
adRNA8
adRNA9
adRNA10
adRNA11
adRNA12
adRNA13
adRNA14
adRNA15
adRNA16
adRNA17
adRNA18
adRNA19
adRNA20
adRNA21
adRNA22
adRNA23
adRNA24

FIGURE 20

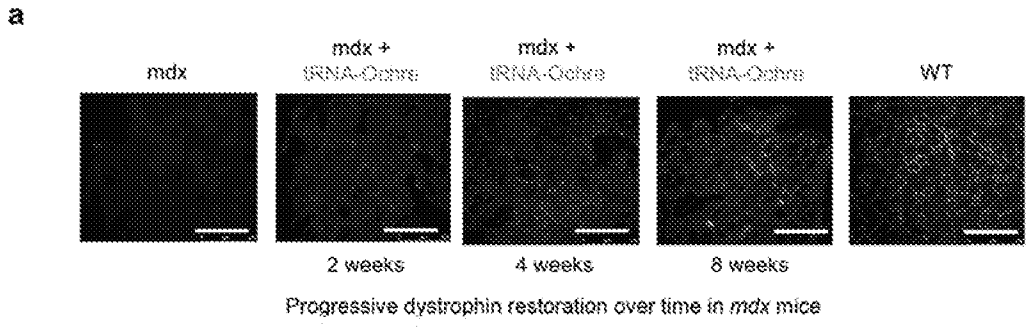

a
mdx
mdx + tRNA-Ochre
mdx + tRNA-Ochre
mdx + tRNA-Ochre
WT
2 weeks
4 weeks
8 weeks
Progressive dystrophin restoration over time in mdx mice
b
mdx + tRNA-UAA -UAA
mdx + tRNA-UAA +UAA
nNOS
c
tRNA + aaRS
mdx
tRNA-Ochre
-UAA
+UAA
Cas9 + gRNAs
WT 2%
WT 10%
Dystrophin (427kDa)
Gapdh (41kDa)

FIGURE 21

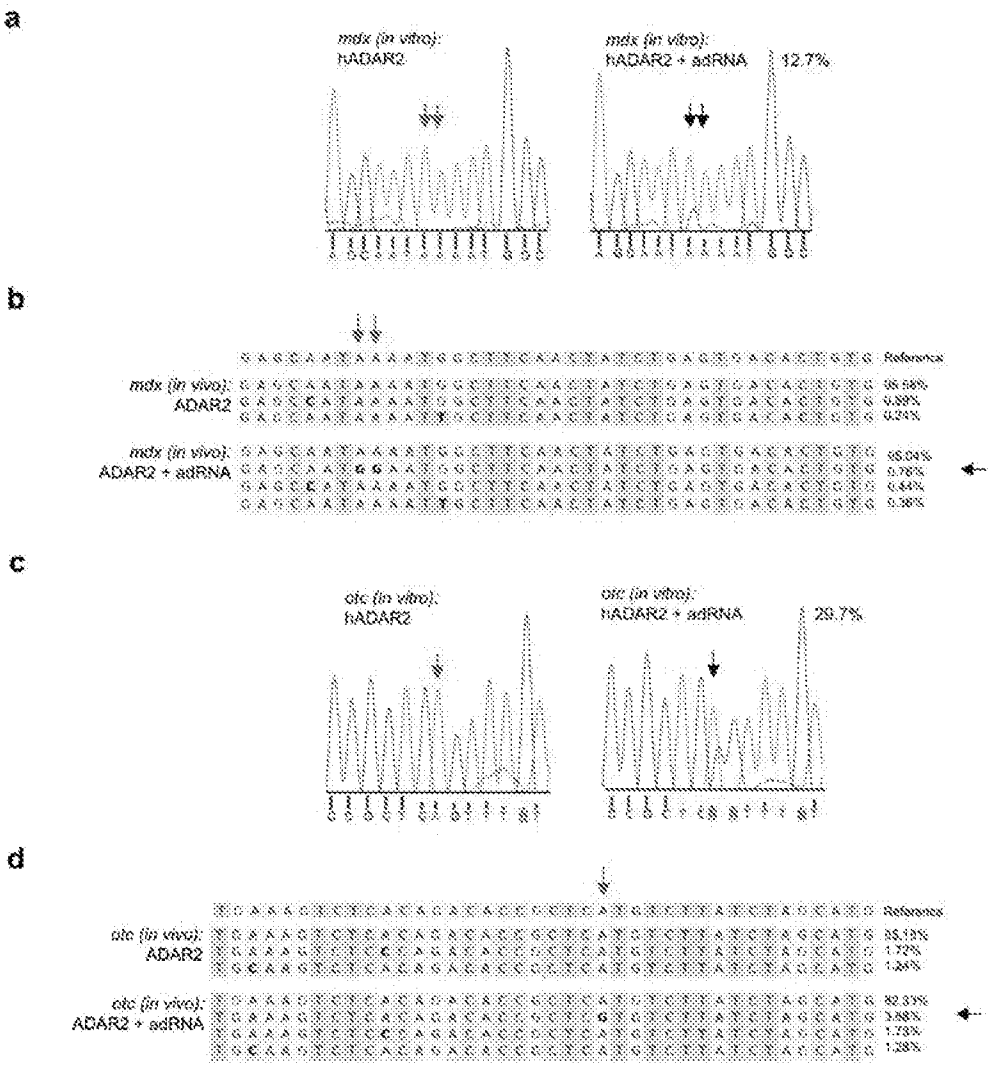

a
mdx (in vitro):
hADAR2
mdx (in vitro):
hADAR2 + adRNA
12.7%
b
Reference
mdx (in vivo):
ADAR2
mdx (in vivo):
ADAR2 + adRNA
c
otc (in vitro):
hADAR2
otc (in vitro):
hADAR2 + adRNA
29.7%
d
Reference
otc (in vivo):
ADAR2
otc (in vivo):
ADAR2 + adRNA

FIGURE 22

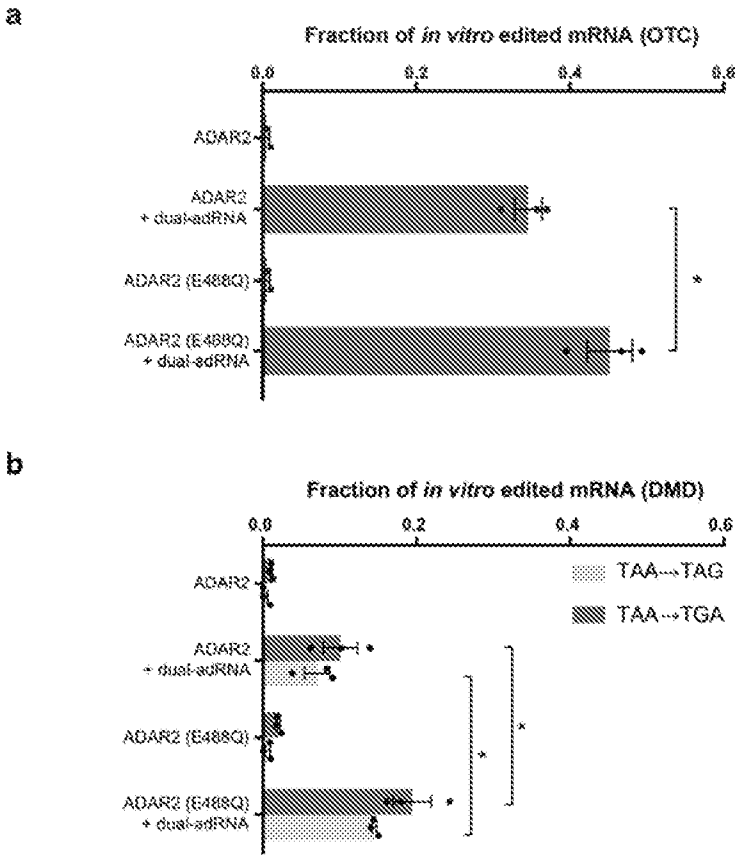
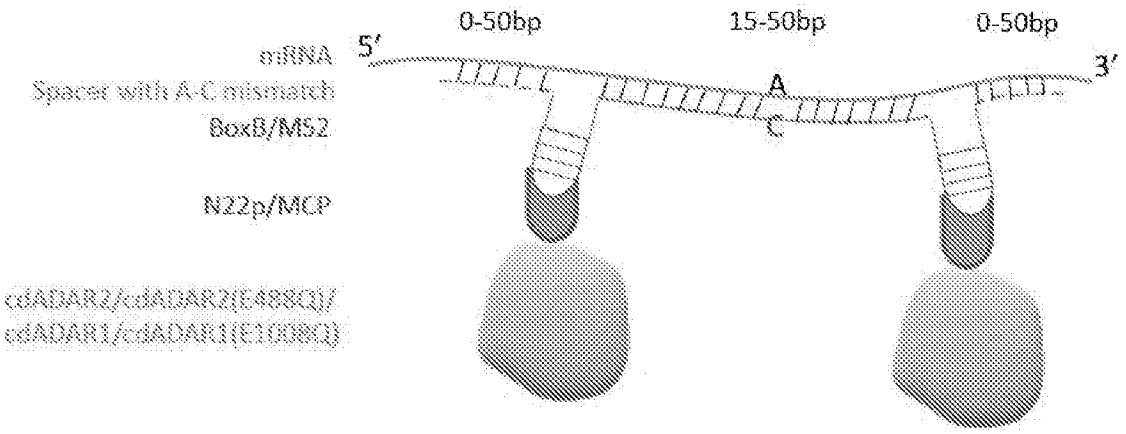

a
Fraction of in vitro edited mRNA (OTC)
0.0
0.2
0.4
0.6
ADAR2
ADAR2 + dual-adRNA
ADAR2 (E488Q)
ADAR2 (E488Q) + dual-adRNA
*
b
Fraction of in vitro edited mRNA (DMD)
0.0
0.2
0.4
0.6
TAA→TAG
TAA→TGA
ADAR2
ADAR2 + dual-adRNA
ADAR2 (E488Q)
ADAR2 (E488Q) + dual-adRNA
*
*
*

FIGURE 23

a


0-50bp
15-50bp
0-50bp
mRNA
5′
3′
Spacer with A-C mismatch
A
C
BoxB/MS2
N22p/MCP
cdADAR2/cdADAR2(E488Q)/
cdADAR1/cdADAR1(E1008Q)

b

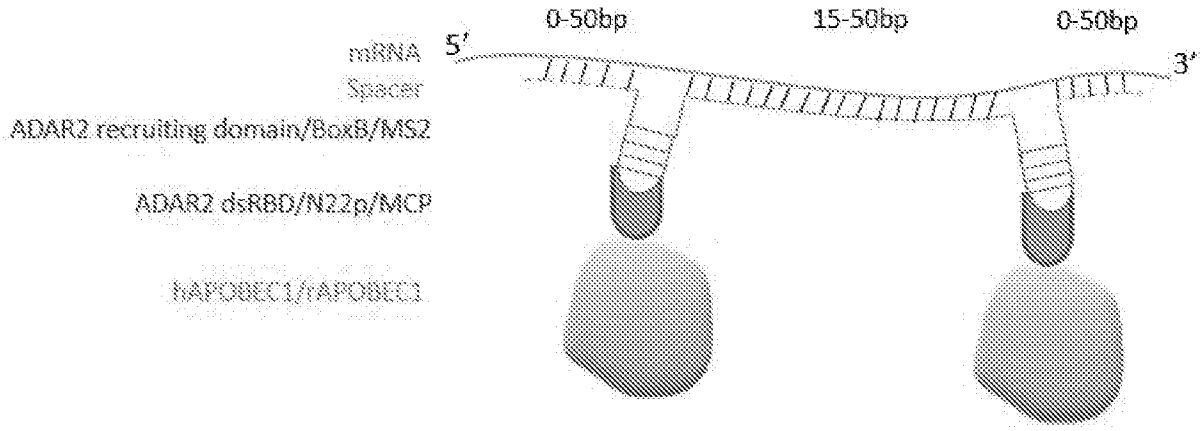
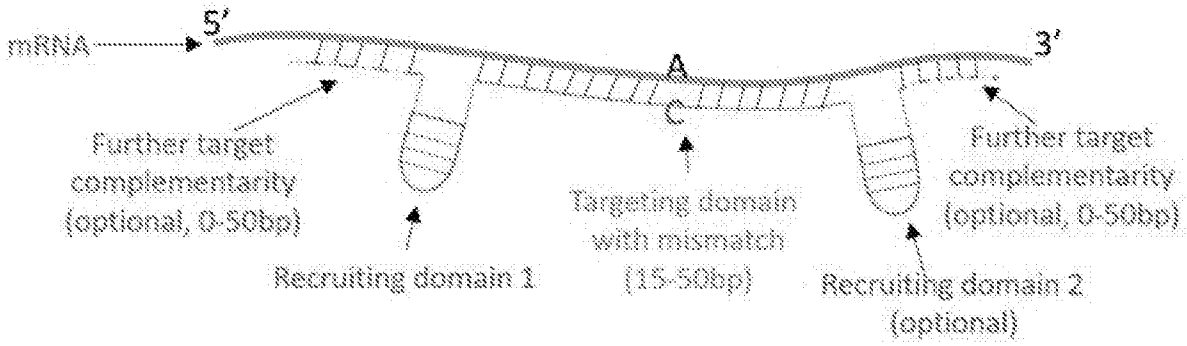

0-50bp
15-50bp
0-50bp
mRNA
5′
3′
Spacer
ADAR2 recruiting domain/BoxB/MS2
ADAR2 dsRBD/N22p/MCP
hAPOBEC1/rAPOBEC1

FIGURE 23 contd.

c



d

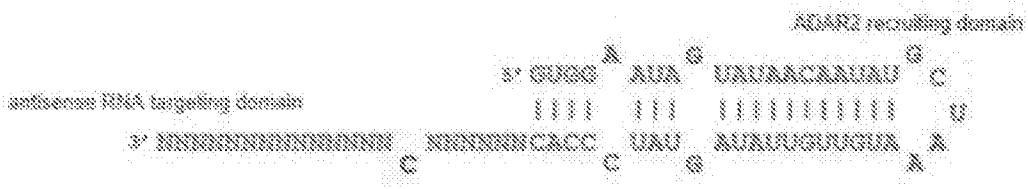
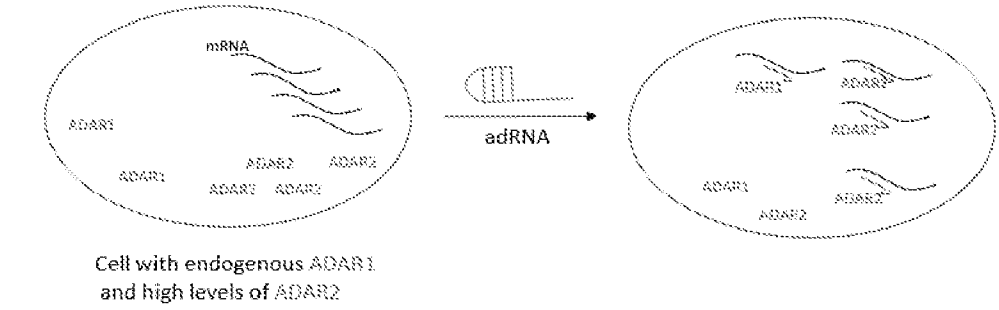


FIGURE 24

a


mRNA
ADAR1
ADAR2
adRNA
Cell with endogenous ADAR1
and high levels of ADAR2

b

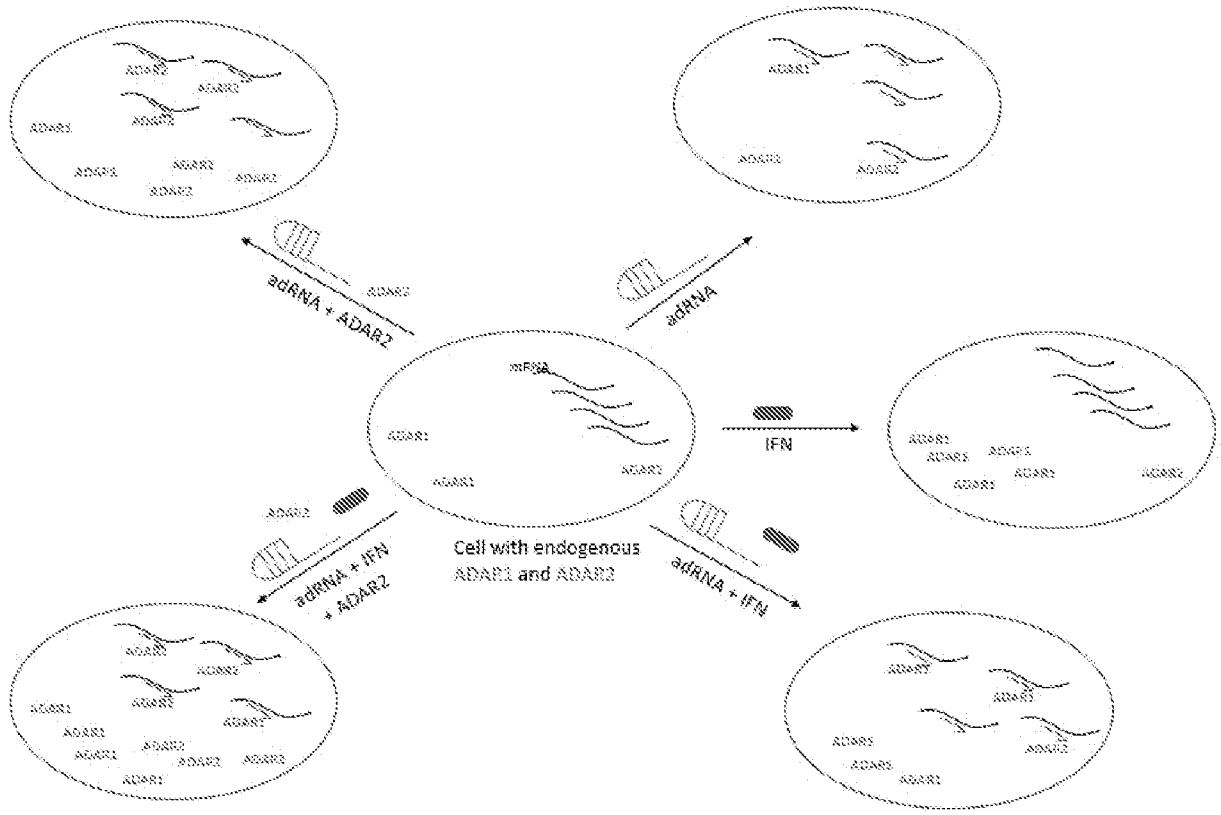

adRNA + ADAR2
adRNA
IFN
adRNA + IFN
+ ADAR2
adRNA + IFN
Cell with endogenous
ADAR1 and ADAR2

FIGURE 25

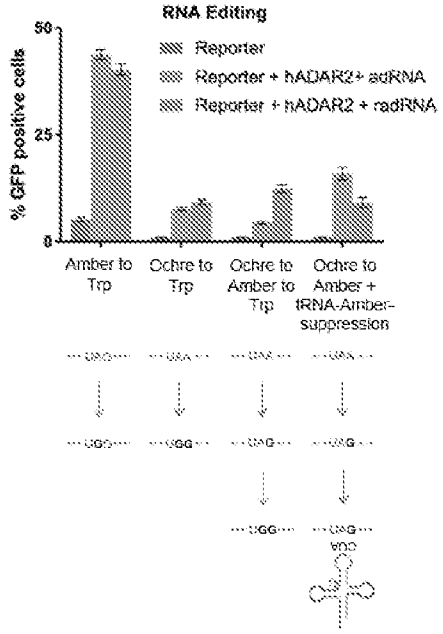

RNA Editing
Reporter
Reporter + hADAR2+ adRNA
Reporter + hADAR2 + radRNA
% GFP positive cells
50
25
0
Amber to Trp
Ochre to Trp
Ochre to Amber to Trp
Ochre to Amber + tRNA-Amber-suppression

FIGURE 26

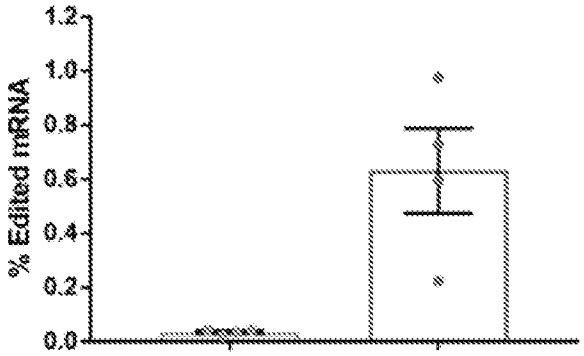


FIGURE 27

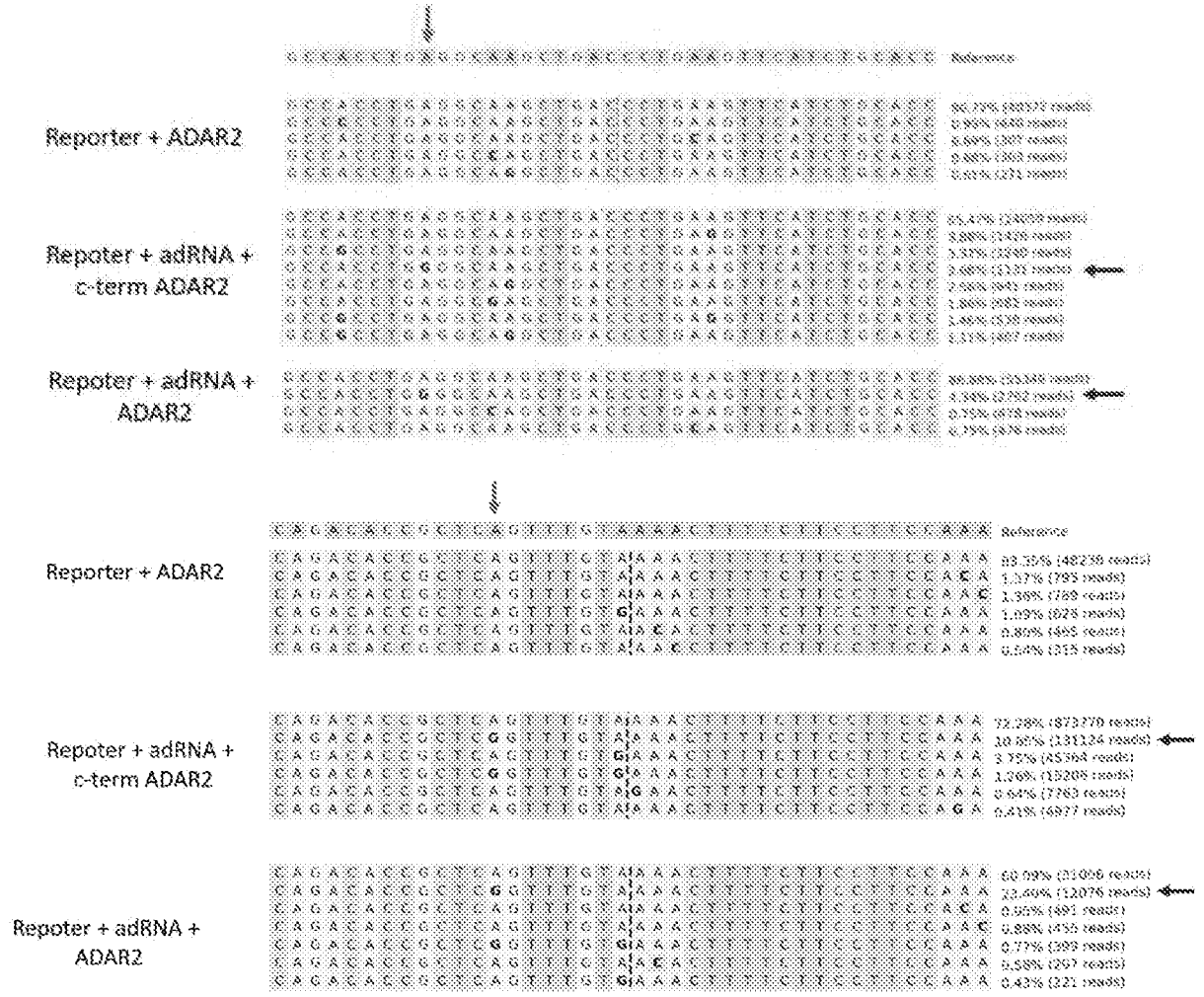

Reporter + ADAR2
Repoter + adRNA + c-term ADAR2
Repoter + adRNA + ADAR2
Reporter + ADAR2
Repoter + adRNA + c-term ADAR2
Repoter + adRNA + ADAR2

FIGURE 28

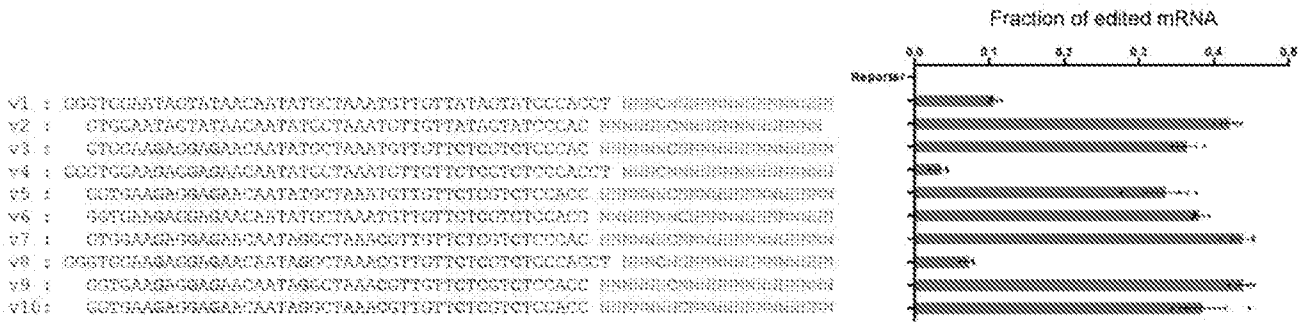
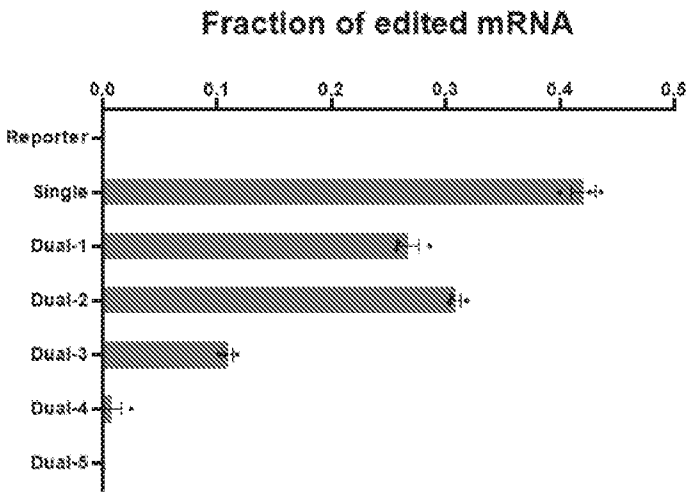

Fraction of edited mRNA
0.0
0.1
0.2
0.3
0.4
0.5
Reporter
v1 :
v2 :
v3 :
v4 :
v5 :
v6 :
v7 :
v8 :
v9 :
v10:

FIGURE 29



Single:

GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACACAAACCGAGCGGTGTCTGT

Dual 1:

GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACCAAACCGAGCGGTGTCTGTGGTGGAATAGTATAACAAT

ATGCTAAATGTTGTTATAGTATCCCAC

Dual 2:

GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACTACAAACCGAGCGGTGTCTGGTGGAATAGTATAACAAT

ATGCTAAATGTTGTTATAGTATCCCAC

Dual 3:

GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACTTTACAAACCGAGCGGTGTCGTGGAATAGTATAACAAT

ATGCTAAATGTTGTTATAGTATCCCAC

Dual 4:

GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACGTTTTACAAACCGAGCGGTGGTGGAATAGTATAACAAT

ATGCTAAATGTTGTTATAGTATCCCAC

Dual 5:

GTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACAAGTTTTACAAACCGAGCGGGTGGAATAGTATAACAAT

ATGCTAAATGTTGTTATAGTATCCCAC

FIGURE 30

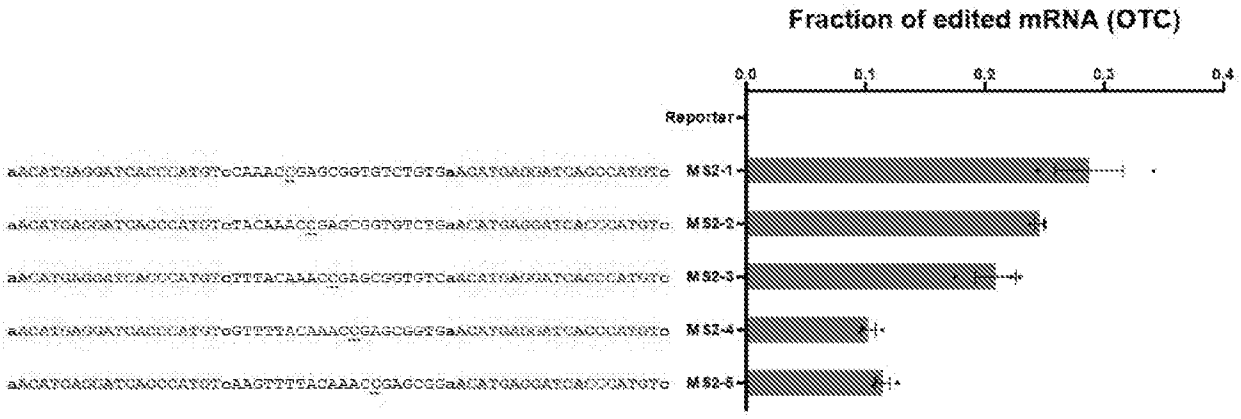
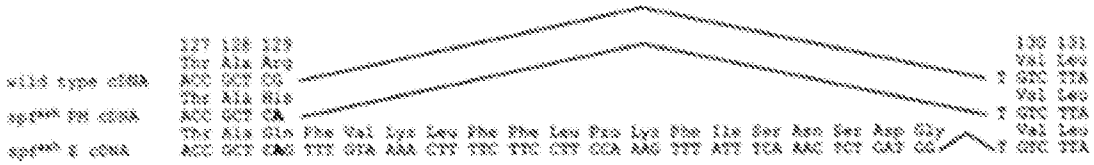

Fraction of edited mRNA (OTC)
0.0
0.1
0.2
0.3
0.4
Reporter
MS2-1
MS2-2
MS2-3
MS2-4
MS2-5

FIGURE 31



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US1 8/20762

A. CLASSIFICATION OF SUBJECT MATTER
IPC - C12Q 1/68; C12N 15/09, 5/10, 15/79, 15/85 (2018.01)
CPC -
C12Q 1/6883; C12N 15/09, 5/10, 15/79, 15/8509

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.
X	US 2002/0156042 A1 (PANCHAL, RG et al.) 24 October, 2002; paragraphs [0010]-[0011], [0013], [0015], [0039], [0069], [0076]; claims 14, 32; figures 1B-1C, 2A	1, 3/1, 4/3/1, 11, 13/11, 14/13/11, 24, 26/24, 27/26/24
Y		2, 3/2, 4/3/2, 5/1-2, 6/5/1-2, 12, 13/12, 14/13/12. 15/11-12, 16/15/11-12. 25. 27/26/25, 28/24-25, 29/28/24-25
Y	(GATTI) SMRT Compounds Correct Nonsense Mutations in Primary Immunodeficiency and Other Genetic Models. Annals of the New York Academy of Sciences. February, 2012; Vol. 1250; pages 1-13; figure 1; page 4, paragraph 3; DOI: 10.1111/J.1749-6632.2012.06467 .x	2, 3/2, 4/3/2, 5/2, 6/5/2, 12, 13/12, 14/13/12, 15/12, 16/15/12, 25, 26/25, 27/26/25, 28/25, 29/28/25
Y	(BIDDLE, W et al.) Modification of Orthogonal tRNAs: Unexpected Consequences for Sense Codon Reassignment. Nucleic Acids Research. 23 October, 2016; Vol. 44, No. 21; pages 10042-10050; abstract; pago 10013, column 1, paragraph ?; page 10044, column 2, paragraph 2; DOI: 10.1093/nar/gkw948	5/1-2, 6/5/1-2, 15/11-12, 16/15/11-12, 28/24-25, 29/28/24-25

☒ 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
"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 internaiional 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 trie 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	Date of mailing of the international search report
17 June 2018 (17.07.2018)	01 AUG 2018

Name and mailing address of the ISA/	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/20762

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,759,833 A (SHIBA, K et al.) 02 June, 1998; claim 9	6/5/1-2, 16/15/1 1-12, 29/28/2 4-25
P, X	(KATREKAR, D et al.) In vivo RNA Targeting of Point Mutations via Suppressor tRNAs and Adenosine Deaminases. BioRxiv. 27 October, 2017; pages 1-25; whole document; DOI: 10.1 101/210278	1-2, 3/1-2, 4/3/1-2, 5/1-2, 6/5/1-2, 11-12, 13/1 1-12, 14/13/1 1-12, 15/1 1-12, 16/15/1 1-12, 24-25, 26/24-25, 27/26/24-25, 28/24-25, 29/28/24-25

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/20762

Box No. I I 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.: 7-10, 17-23, 30-34, 45-52, 63-73, 84-92
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. Ill 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:

-" *- Please See Within the Next Supplemental Page-*"-

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 oiily 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.:
firoup 1 Claims 1-6, 11-16 and 24-29

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
Information on patent family members

International application No.
PCT/US 18/20762

-'"-Continued from Box 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 examined, the appropriate additional examination fees must be paid.

Group I, Claims 1-6, 11-16 and 24-29 are directed toward a method for restoring expression of a protein comprising a point mutation, comprising administering to a subject a vector encoding one or more tRNA having an anticodon sequence that recognizes a codon comprising the point mutation; a vector therefor; and a method of treating a disease associated therewith.

Group II, Claims 35-44, 53-62, and 74-83 are directed toward a method for restoring expression of a protein comprising a point mutation in an RNA sequence encoding the protein comprising administering one or more vectors encoding an ADAR based RNA editing system comprising one or more forward guide RNAs for the ADAR ("adRNAs") and one or more corresponding reverse guide RNAs for the ADAR ("radRNAs") to a subject; and a recombinant expression system and method of treating a disease associated therewith.

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: the special technical features of Group I include a tRNA having an anticodon that recognizes a codon comprising a point mutation, not present in Group II; the special technical features of Group II include an ADAR based RNA editing system, not present in Group I.

Groups I and II share the technical features including: a method for restoring expression of a protein comprising a point mutation in an RNA sequence encoding the protein in a subject in need thereof comprising administering to the subject an encoding vector; a method of treating a disease, disorder, or condition characterized by the presence of a point mutation in an RNA sequence encoding a gene associated with the disease, disorder, or condition in a subject in need thereof comprising administering to the subject an encoding vector.

However, these shared technical features are previously disclosed by US 6,309,830 B1 to Panchal et al. (hereinafter 'Panchal').

Panchal discloses a method for restoring expression of a protein comprising a point mutation in an RNA sequence encoding the protein (completing translation of a protein product from a sequence comprising a nonsense mutation (a method for restoring expression of a protein comprising a point mutation in an RNA sequence encoding the protein); column 3, lines 21-30) in a subject in need thereof (in a patient (subject) with XP (in need thereof); column 14, line 61 - column 15, line 50) comprising administering to the subject an encoding vector (comprising administering to the subject an encoding vector; column 4, lines 1-12, column 21, lines 23-28); and a method of treating a disease, disorder, or condition characterized by the presence of a point mutation in an RNA sequence encoding a gene associated with the disease, disorder, or condition (a method of treating Xeroderma Pigmentosa (a disease, disorder, or condition characterized by the presence of a point mutation in an RNA sequence encoding a gene associated with the disease, disorder, or condition); column 4, lines 1-12. column 14, line 61- column 15, line 50) in a subject in need thereof (in a patient (subject) with XP (in need thereof); column 14, line 61 - column 15, line 50) comprising administering to the subject an encoding vector (comprising administering to the subject an encoding vector; column 4, lines 1-12, column 21, lines 23-28).

Since none of the special technical features of the Groups I and II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Panchal reference, unity of invention is lacking.