17] including cell type specific delivery, the efficiency of homologous recombination, and the frequency of on-target repair errors and off-target editing. Moreover, at >6 kb per transcript, the sodium channel coding region of *SCN1A* exceeds the capacity of AAV thus limiting the utility of the rapid pace of advancing AAV therapies for tissue specific gene replacement. Thus, there are limited clinical options for *SCN1A* LOF associated with Dravet Syndrome, and the therapeutic pipeline remains thin. The compositions and methods described herein address this and other deficiencies in the art.

SUMMARY

The compositions and methods described herein have a broad range of applications, providing potential disease modifying therapies for a range of haploinsufficiency disorders. The key advantage of this mRNA regulon approach is that it is 100% agnostic to mutation type and location by stabilizing the mRNA of the wild-type (WT) allele. The potential to reengineer these approaches for other indications is high and facile.

Thus, provided herein are fusion proteins comprising: an RNA effector protein that targets mRNA(s) of an active allele of a gene associated with a haploinsufficiency disorder; and a regulon moiety that stimulates and/or stabilizes the mRNA(s). In one aspect, the present disclosure refers to a fusion protein comprising: an RNA effector protein that targets mRNA(s) of an active allele of a gene associated with a haploinsufficiency disorder; and a regulon moiety that stimulates and/or stabilizes the mRNA(s).

In some examples, the RNA effector protein is a Cas effector protein selected from the group consisting of Cas9, Cas12, Cas13, and Cas14. In some examples, the RNA effector protein is Cas13b. In some examples, the Cas effector protein is a catalytically inactive Cas protein.

In some examples, the regulon moiety is PABPC1 or NAT10.

In some examples, the fusion proteins further comprise a linker and/or a spacer.

In some examples, the fusion proteins further comprise a nuclear export signal and/or an epitope tag.

In some examples, the RNA effector protein is N terminal to the regulon moiety. In some examples, the RNA effector protein is C terminal to the regulon moiety.

In some examples, the fusion proteins comprise or consist of SEQ ID NO: 48 or SEQ ID NO: 49 or a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO: 48 or SEQ ID NO: 49.

Also provided herein are polynucleotides encoding any one of the fusion proteins described herein. In one aspect, the present disclosure refers to a polynucleotide encoding the fusion protein as described herein. Also provided herein are vectors comprising any one of the polynucleotides described herein. In another aspect, the present disclosure refers to a vector comprising the polynucleotide as described herein. Also provided herein are cells comprising any one of the vectors described herein. In yet another aspect, the present disclosure refers to a cell comprising the vector as described herein.

Also provided herein are systems comprising: a fusion protein comprising: an RNA effector protein that targets mRNA(s) of an active allele of a gene associated with a haploinsufficiency disorder; and a regulon moiety that stimulates and/or stabilizes the mRNA(s); and a gRNA that forms a complex with the RNA effector protein and comprises a complementarity region that hybridizes with the mRNA(s) of the active allele. In one aspect, the present disclosure refers to a system comprising: a fusion protein comprising: an RNA effector protein that targets mRNA(s) of an active allele of a gene associated with a haploinsufficiency disorder; and a regulon moiety that stimulates and/or stabilizes the mRNA(s); and a gRNA that forms a complex with the RNA effector protein and comprises a complementarity region that hybridizes with the mRNA(s) of the active allele.

In some examples, the RNA effector protein is a Cas effector protein selected from the group consisting of Cas9, Cas12, Cas13, and Cas14. In some examples, the Cas effector protein is Cas13b. In some examples, the Cas effector protein is a catalytically inactive Cas effector protein.

In some examples, the regulon moiety is PABPC1 or NAT10.

In some examples, the fusion protein further comprises a linker and/or a spacer. In some examples, the fusion protein further comprises a nuclear export signal and/or an epitope tag.

In some examples, the RNA effector protein is N terminal to the regulon moiety. In some examples, the RNA effector protein is C terminal to the regulon moiety.

In some examples, the fusion protein comprises or consists of SEQ ID NO: 48 or SEQ ID NO: 49 or a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO: 48 or SEQ ID NO: 49.

In some examples, the gRNA targets an mRNA encoding MeCP2, SCN1A, SYNGAP1, SHANK3, CHD2, or PTEN. In some examples, the gRNA comprises a complementarity region designed to hybridize to an mRNA selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 , SEQ ID NO: 17, SEQ ID NO: 18, and combinations thereof. In some examples, the gRNA comprises a complementarity region designed to hybridize to an mRNA selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 15, SEQ ID NO: 17, and combinations thereof.

In some examples, the gRNA comprises a complementarity region designed to hybridize to an mRNA encoding an amino acid selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, and combinations thereof.

In some examples, the gRNA comprises a complementarity region designed to hybridize to an mRNA selected from the group consisting of SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID

NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, and combinations thereof.

In some examples, the gRNA comprises a complementarity region designed to hybridize to an mRNA encoding an amino acid selected from the group consisting of SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, and combinations thereof.

In some examples, the gRNA is selected from the group consisting of SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, and SEQ ID NO: 72.

Also provided herein are one or more polynucleotide(s) encoding any one of the systems described herein. In one aspect, the present disclosure refers to one or more polynucleotide(s) encoding the system as described herein. Also provided herein are one or more vector(s) comprising any one of the polynucleotide(s) described herein. In another aspect, the present disclosure refers to one or more vector(s) comprising the polynucleotide(s) as described herein. Also provided herein are cells comprising any one of the vector(s) described herein. In yet another aspect, the present disclosure refers to a cell comprising the vector(s) as described herein.

Also provided herein are complexes comprising: a fusion protein comprising: an RNA effector protein that targets mRNA(s) of an active allele of a gene associated with a haploinsufficiency disorder; and a regulon moiety that stimulates and/or stabilizes the mRNA(s), bound to a gRNA comprising a complementarity region that hybridizes with the mRNA(s) of the active allele. In one aspect, the present disclosure refers to a complex comprising: a fusion protein comprising: an RNA effector protein that targets mRNA(s) of an active allele of a gene associated with a haploinsufficiency disorder; and a regulon moiety that stimulates and/or stabilizes the mRNA(s), bound to a gRNA comprising a complementarity region that hybridizes with the mRNA(s) of the active allele.

In some examples, the RNA effector protein is dCas13b and the regulon moiety is PABP1 or NAT10.

In some examples, the gRNA comprises a complementarity region designed to hybridize to an mRNA selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 , SEQ ID NO: 17, SEQ ID NO: 18, and combinations thereof. In some examples, the gRNA comprises a complementarity region designed to hybridize to an mRNA selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 15, SEQ ID NO: 17, and combinations thereof.

In some examples, the gRNA comprises a complementarity region designed to hybridize to an mRNA encoding an amino acid selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, and combinations thereof.

In some examples, the gRNA comprises a complementarity region designed to hybridize to an mRNA selected from the group consisting of SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, and combinations thereof.

In some examples, the gRNA comprises a complementarity region designed to hybridize to an mRNA encoding SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, and combinations thereof.

Also provided herein are complexes comprising: a fusion protein comprising: an RNA effector protein that targets mRNA(s) of an active allele of a gene associated with a haploinsufficiency disorder; and a regulon moiety that stimulates and/or stabilizes the mRNA(s), bound to a gRNA and the mRNA. In one aspect, the present disclosure refers to a complex comprising: a fusion protein comprising: an RNA effector protein that targets mRNA(s) of an active allele of a gene associated with a haploinsufficiency disorder; and a regulon moiety that stimulates and/or stabilizes the mRNA(s), bound to a gRNA and the mRNA.

In some examples, the RNA effector protein is dCas13b and the regulon moiety is PABP1 or NAT10.

In some examples, the mRNA is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 , SEQ ID NO: 17, SEQ ID NO: 18, and combinations thereof. In some examples, the mRNA encodes an amino acid selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 15, SEQ ID NO: 17, and combinations thereof.

In some examples, the mRNA is selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, and combinations thereof.

In some examples, the mRNA is selected from the group consisting of SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, and combinations thereof.

In some examples, the mRNA encodes an amino acid selected from the group consisting of is selected from the group consisting of SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, and combinations thereof.

Also provided herein are pharmaceutical compositions comprising: any one of the fusion proteins or systems described herein. In some examples, the pharmaceutical compositions further comprise a pharmaceutically acceptable carrier.

In one aspect, the present disclosure refers to a pharmaceutical composition comprising: the fusion protein or system as described herein.

Also provided herein are pharmaceutical compositions comprising: one or more nucleic acids encoding any one of the fusion proteins or systems described herein. In one aspect, the present disclosure refers to a pharmaceutical composition comprising: one or more nucleic acids encoding the fusion protein or system as described herein.

Also provided herein are viral vectors comprising one or more nucleic acids encoding any one of the fusion proteins or systems described herein. In one aspect, the present disclosure refers to a viral vector comprising one or more nucleic acids encoding the fusion protein or system as described herein. In some examples, the viral vector is an adeno-associated viral vector.

Also provided herein are nanoparticles or liposomes comprising any one of the fusion proteins or systems described herein or one or more nucleic acids encoding any one of the fusion proteins or systems described herein. In one aspect, the present disclosure refers to a nanoparticle or liposome comprising the fusion protein or system as described herein or one or more nucleic acids encoding the fusion protein or system as described herein.

Also provided herein are methods of stimulating or stabilizing mRNA(s), the method comprising: contacting the mRNA(s) with any one of the fusion proteins or systems described herein. In some examples, the method is carried out, in vitro, in vivo, or ex vivo. In one aspect, the present disclosure refers to an *in vitro* method of stimulating or stabilizing mRNA(s), the method comprising: contacting the mRNA(s) with the fusion protein or system as described herein. In another aspect, the present disclosure refers to use of the fusion protein or system as described herein in the manufacture of a medicament for stimulating or stabilizing mRNA(s), wherein the medicament is to be contacted with the mRNA(s) *in vivo* or *ex vivo*.

Also provided herein are methods of treating or preventing a haploinsufficiency disorder in a subject, the method comprising: administering to the subject a fusion protein or a nucleic acid encoding a fusion protein comprising: an RNA effector protein that targets mRNA(s) of an active allele of a gene associated with a haploinsufficiency disorder; and a regulon moiety that stimulates and/or stabilizes the mRNA(s); and a gRNA or a nucleic acid encoding a gRNA designed to form a complex with the RNA effector protein and comprising a complementarity region designed to hybridize with the mRNA of the active allele. In one aspect, the present disclosure refers to use of a fusion protein or a nucleic acid encoding a fusion protein comprising: an RNA effector protein that targets mRNA(s) of an active allele of a gene associated with a haploinsufficiency disorder; and a regulon moiety that stimulates and/or stabilizes the mRNA(s); and a gRNA or a nucleic acid encoding a gRNA designed to form a complex with the RNA effector protein and comprising a complementarity region designed to hybridize with the mRNA of the active allele in the manufacture of a medicament for treating or preventing a haploinsufficiency disorder in a subject.

In some examples, the RNA effector protein is a Cas effector protein selected from the group consisting of a Cas9, Cas12, Cas13, and Cas14. In some examples, the Cas effector protein is Cas13b. In some examples, the Cas effector protein is a catalytically inactive Cas protein.

In some examples, the regulon moiety is PABPC1 or NAT10.

In some examples, the fusion protein further comprises a linker and/or a spacer. In some examples, the fusion protein further comprises a nuclear export signal and/or an epitope tag.

In some examples, the RNA effector protein is N terminal to the regulon moiety. In some examples, the RNA effector protein is C terminal to the regulon moiety.

In some examples, the fusion protein comprises or consists of SEQ ID NO: 48 or SEQ ID NO: 49 or a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO: 48 or SEQ ID NO: 49.

In some examples, the haploinsufficiency disorder is selected from the group consisting from 5qsyndrome,Adams-Oliver syndrome 1, Adams-Oliver syndrome 3, Adams-Oliver syndrome 5,Adams-Oliver syndrome 6, Alagille syndrome 1, Autoimmune lymphoproliferative syndrome type IA, Autoimmune lymphoproliferative syndrome type V,

Autosomal dominant deafness-2A,Brain malformations with or without urinary tract defects (BRMUTD), Carney complex type 1,CHARGE syndrome, Cleidocranial dysplasia, Currarino syndrome, Denys-Drash syndrome/Frasier syndrome, Developmental delay, intellectual disability, obesity, and dysmorphic features(DIDOD), DiGeorge syndrome (TBXI-associated), Dravet syndrome, Duane-radial raysyndrome, Ehlers-Danlos syndrome (classic-like), Ehlers-Danlos syndrome (vascular type),Feingold syndrome 1, Frontotemporal lobar degeneration with TDP43 inclusions (FTLD-TDP),GRN-related, GLUT I deficiency syndrome, Greig cephalopolysyndactyly syndrome, Hereditary hemorrhagic telangiectasia type 1, Holoprosencephaly 3, Holoprosencephaly 4,Holoprosencephaly 5, Holt-Oram syndrome, Hypoparathyroidism, sensorineural deafness, andrenal disease (HDR), Kleefstra syndrome 1, Klippel-Trenaunay syndrome (AAGF-related), Leri-Weill dyschondrosteosis, Marfan syndrome, Mental retardation and distinctive facial features with or without cardiac defects (MRFACD), Mental retardation, autosomal dominant 1, Mental retardation, autosomal dominant 19, Mental retardation, autosomal dominant 29, Nail-patella syndrome (NPS), Phelan-McDermid syndrome, Pitt-Hopkins syndrome, Primary pulmonary hypertension 1, Rett syndrome (congenital variant), Smith-Magenis syndrome (RAII associated), Sotos syndrome 1, Sotos syndrome 2, Stickler syndrome type I, Supravalvular aorticstenosis, SYNGAPI-related intellectual disability, Treacher Collins syndrome, Trichorhinophalangeal syndrome type I, Ulnar-mammary syndrome, van der Woude syndrome1, Waardenburg syndrome type 1, W aardenburg syndrome type 2A, and Waardenburg syndrometype 4C.

In some examples, the haploinsufficiency disorder is a CNS haploinsufficiency disorder. In some examples, the CNS haploinsufficiency disorder is selected from the group consisting of episodic ataxia, familial hemiplegia migraine, CDKL5 deficiency disorder, CHD2 myoclonic encephalopathy, familial focal epilepsy with variable loci, FOXG1 syndrome, benign familial neonatal seizures, Rett syndrome, Dravat syndrome, SCN2A-epileptic encephalopathy, SCN2A-developmental encephalopathy, SCN8A-epileptic encephalopathy, SC8A familial infantile epilepsy, early infantile epileptic encephalopathy, myoclonic-atonic epilepsy, early infantile epileptic encephalopathy, SYNGAP1-related intellectual disability, tuberous sclerosis, Lennox-Gastaut Syndrome, FoxG1 syndrome, KCNQ2-related epileptic encephalopathy, PCDH19-related epilepsy, SLC6A1-related myoclonic-astatic epilepsy, STXBP1-related epileptic encephalopathy, SYNGAP1 syndrome, and combinations thereof. In some examples, the haploinsufficiency disorder is Dravet Syndrome or Rett syndrome.

In some examples, the subject has a haploinsufficiency in a gene selected from the group consisting of AGGFI, ARHGAP31, BMPR2, CHD7, COL2Al, COL3Al, CTLA4, CTNNBI, DLL4, EHMTI, ELN,ENG, FAS, FBNI, FOXGI, GATA3, GLI3, GRN, IRF6, JAGI,

KCNQ4, LMXIB, MBD5,MED13L, MITF, MNXI, MYCN, NFIA, NFIX, NOTCH!, NSDI, PAX3, PHIP, PRKARIA,RAil, RBPJ, RPS14, RUNX2, SALL4, SCNIA, SETBPI, SHANK3, SHH, SHOX,SLC2Al/GLUT1, SOXI0, SYNGAPI, TBXI, TBX3, TBX5, TCF4, TCOFI, TGIFI, TNXB,TRPSI, WTI, ZIC2, and combinations thereof. In some examples, the subject has a haploinsufficiency in a gene selected from the group consisting of SCN1A, SCN2A, SCN8A, SCN12A5, SPTAN1, CDKL5, CHD2, FOXG1, KCNQ2, PCDH19, SLC6A1, STXBP1, SYNGAP1, CACNA1A, DEPDC5, MECP2, TSC1, TSC2, and combinations thereof.

In some examples, the subject has mutation selected from the list in Table 4 and combinations thereof. In some examples, the subject has a mutation selected from the list in Table 6 and combinations thereof. In some examples, the subject is a mammal. In some examples, the subject is a human.

In some examples, the fusion protein and gRNA are administered as part of a pharmaceutical composition. In some examples, administering comprises administering a viral vector comprising nucleic acid sequence(s) encoding the fusion protein and gRNA to the subject. In some examples, administering comprises administering a nanoparticle or liposome comprising the fusion protein and gRNA or nucleic acid sequence(s) encoding the fusion protein and gRNA to the subject.

Throughout this application, various examples may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosure. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

As used in the specification and claims, the singular forms “a”, “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a sample” includes a plurality of samples, including mixtures thereof.

The terms “determining,” “measuring,” “evaluating,” “assessing,” “assaying,” and “analyzing” are often used interchangeably herein to refer to forms of measurement. The terms include determining if an element is present or not (for example, detection). These terms can include quantitative, qualitative or quantitative and qualitative determinations. Assessing can be relative or absolute. “Detecting the presence of” can include determining the amount of

something present in addition to determining whether it is present or absent depending on the context.

As used herein, the term "about" a number refers to that number plus or minus 10% of that number. The term "about" a range refers to that range minus 10% of its lowest value and plus 10% of its greatest value.

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 disclosure belongs. Methods and materials are described herein for use in the present disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the disclosure will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

FIG. 1 is a schematic showing SCN1A topology and premature-termination codons (PTC). Each of the 182 SCN1A PTCs is show by color.

FIG. 2 is a schematic representing the tethered function assay. The fused functional protein to the tethered protein bind to the mRNA of interest to enhance or stabilize the mRNA.

FIG. 3A is a schematic showing the experimental approach applied for this assay. Briefly, PABPC1 was fused to dCas13b tethering protein and guided to 3'UTR of the Luciferase gene via designed guide RNAs(gRNA) to enhance its translation.

FIG. 3B shows a firefly luciferase assessment depicting about 2-folds increase in PAB-tethered luciferase targeting over non-targeting in HEK-293 cells.

FIG. 4 shows a map of a plasmid pJC1211 encoding a Cas13b-PABPC1 fusion protein.

FIGS. 5A–5AB show the sequence and features of the plasmid pJC1211 (SEQ ID NO: 50). The nucleic acid sequence is shown in both 5'->3' and the reverse complement (3'->5').

FIG. 6 is a map of plasmid pJC1212 encoding a luciferase targeting Cas13b gRNA.