

CLAIMS

1. An isolated nucleic acid comprising a transgene configured to express at least one DNA binding domain fused to at least one transcriptional regulator domain, wherein the at least one DNA binding domain binds to a SCN1A target-gene or a regulatory region of a SCN1A target-gene, ~~and~~ wherein the SCN1A target-gene encodes a voltage-gated sodium channel, and wherein the at least one DNA binding domain binds to a nucleic acid sequence set forth in any one of SEQ ID NOs: 5-7.

2. The isolated nucleic acid of claim 1, wherein the transgene is flanked by inverted terminal repeats (ITRs) derived from adeno-associated virus (AAV).

3. The isolated nucleic acid of claim 1 or 2, wherein the at least one transcriptional regulator domain upregulates the expression of the SCN1A target gene.

4. The isolated nucleic acid of any one of claims 1-3, wherein the at least one DNA binding domain binds to an untranslated region of the SCN1A target gene, optionally wherein the untranslated region is an enhancer, a promoter, an intron, and/or a repressor; and/or wherein the at least one DNA binding domain binds between 2-2000 bp upstream or between 2-2000 bp downstream of a regulatory region of the SCN1A target gene.

5. The isolated nucleic acid of any one of claims 1-4, wherein the at least one DNA binding domain encodes a zinc finger protein (ZFP), a transcription-activator like effectors (TALE), a dCas protein, and/or a homeodomain.

6. The isolated nucleic acid of any one of claims 1-5, wherein:
(i) ~~the at least one DNA binding domain binds to a nucleic acid sequence set forth in any one of SEQ ID NOs: 5-7; and/or~~
(ii) ~~the at least one DNA binding domain is a zinc finger protein comprising a recognition~~
helix encoded by a nucleic acid having a sequence set forth in any one of SEQ ID NOs: 11-16, 23-28, or 35-40.

7. The isolated nucleic acid of claim 6, wherein:

(a) the at least one DNA binding domain is a zinc finger protein comprising a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 11, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 12, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 13, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 14, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 15, and/or a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 16; or

(b) the at least one DNA binding domain is a zinc finger protein comprising a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 23, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 24, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 25, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 26, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 27, and/or a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 28; or

(c) the at least one DNA binding domain is a zinc finger protein comprising a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 35, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 36, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 37, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 38, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 39, and/or a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 40.

8. The isolated nucleic acid of any one of claims 1-7, wherein the at least one DNA binding domain is a zinc finger protein comprising the amino acid sequence set forth in any one of SEQ ID NOs: 17-22, 29-34, or 41-46.

9. The isolated nucleic acid of claim 8, wherein:

(a) the at least one DNA binding domain is a zinc finger protein comprising a recognition helix comprising SEQ ID NO: 17, a recognition helix comprising SEQ ID NO: 18, a recognition helix comprising SEQ ID NO: 19, a recognition helix comprising SEQ ID NO: 20, a recognition helix comprising SEQ ID NO: 21, and/or a recognition helix comprising SEQ ID NO: 22; or

(b) the at least one DNA binding domain is a zinc finger protein comprising a recognition helix comprising SEQ ID NO: 29, a recognition helix comprising SEQ ID NO: 30, a recognition helix comprising SEQ ID NO: 31, a recognition helix comprising SEQ ID

NO: 32, a recognition helix comprising SEQ ID NO: 33, and/or a recognition helix comprising SEQ ID NO: 34; or

(c) the at least one DNA binding domain is a zinc finger protein comprising a recognition helix comprising SEQ ID NO: 41, a recognition helix comprising SEQ ID NO: 42, a recognition helix comprising SEQ ID NO: 43, a recognition helix comprising SEQ ID NO: 44, a recognition helix comprising SEQ ID NO: 45, and/or a recognition helix comprising SEQ ID NO: 46.

10. The isolated nucleic acid of any one of claims 1-5, wherein the at least one DNA binding domain is a dCas protein, optionally a dCas9 protein, and wherein the isolated nucleic acid further comprises at least one guide nucleic acid, optionally wherein the guide nucleic acid comprises a spacer sequence that targets *SCN1A*.

11. The isolated nucleic acid of claim 10, wherein the guide nucleic acid comprises a spacer sequence having a nucleotide sequence of any one of SEQ ID NOs: 85, 86, 89, 90, 93, or 94.

12. The isolated nucleic acid of ~~any one of claims 10-11~~ claim 10 or 11, wherein the guide nucleic acid comprises a nucleotide sequence of any one of SEQ ID NOs: 83-94.

13. The isolated nucleic acid of any one of claims 1-12, wherein the at least one transcriptional regulator domain comprises a VPR, Rta, p65, or Hsf1 transactivator, or any combination thereof.

14. The isolated nucleic acid of any one of claims 1-13, wherein the at least one ~~transactivation domain~~ transcriptional regulator domain is encoded by the nucleic acid sequence set forth in SEQ ID NO: 47 and/or the at least one transactivation domain is encoded by the amino acid sequence set forth in SEQ ID NO: 48.

15. The isolated nucleic acid of any of claims 1-14, wherein the nucleic acid comprises an AAV2 ITR, optionally wherein the ITR is a Δ TR and/or a mTR.

16. The isolated nucleic acid of any one of claims 1-16, wherein the transgene is operably linked to a promoter, optionally wherein the promoter is a tissue-specific promoter, optionally

wherein the promoter is a neuronal promoter such as SST, NPY, Phosphate-activated glutaminase (PAG), Vesicular glutamate transporter-1 (VGLUT1), Glutamic acid decarboxylase 65 and 57 (GAD65, GAD67), Synapsin I, a-CamKII, Dock10, Prox1, Parvalbumin (PV), Somatostatin (SST), Cholecystokinin (CCK), Calretinin (CR), or Neuropeptide Y (NPY).

17. The isolated nucleic acid of any one claims 1-16, wherein the at least one DNA binding domain is/are fused to the at least one transcriptional regulator domain by a linker domain, optionally wherein the linker domain is (i) a flexible linker, optionally comprised of glycines, or (ii) a cleavable linker.

18. The isolated nucleic acid of any one of claims 1-17, wherein:

the transgene encodes 1 DNA binding domain, 2 DNA binding domains, 3 DNA binding domains, 4 DNA binding domains, 5 DNA binding domains, 6 DNA binding domains, 7 DNA binding domains, 8 DNA binding domains, 9 DNA binding domains, or 10 DNA binding domains; and/or

the transgene encodes 1 transcriptional regulator domain, 2 transcriptional regulator domains, 3 transcriptional regulator domains, 4 transcriptional regulator domains, 5 transcriptional regulator domains, 6 transcriptional regulator domains, 7 transcriptional regulator domains, 8 transcriptional regulator domains, 9 transcriptional regulator domains, or 10 transcriptional regulator domains.

19. A recombinant AAV (rAAV) comprising:

(i) a nucleic acid comprising a transgene encoding at least one DNA binding domain fused to at least one transcriptional regulator domain, wherein the at least one DNA binding domain binds to ~~a-target~~an *SCN1A* gene or a regulatory region of ~~a-target~~the *SCN1A* gene, wherein the *SCN1A*~~target~~-gene encodes a voltage-gated sodium channel, and wherein the at least one DNA binding domain binds to a nucleic acid sequence set forth in any one of SEQ ID NOs: 5-7; and

(ii) at least one capsid protein.

20. The rAAV of claim 19, wherein the transgene is flanked by inverted terminal repeats (ITRs) derived from adeno-associated virus (AAV) and/or the at least one transcriptional regulator domain upregulates the expression of the *SCN1A*~~target~~ gene and/or the at least one

DNA binding domain binds to an untranslated region of the SCN1A~~target~~-gene, optionally wherein the untranslated region is an enhancer, a promoter, an intron, and/or a repressor; and/or the at least one DNA binding domain binds between 2-2000 bp upstream or between 2-2000 bp downstream of a regulatory region of the ~~target~~-SCN1A gene.

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21. The rAAV of claim 19 or 20, wherein the at least one DNA binding domain encodes a zinc finger protein (ZFP), a transcription-activator like effectors (TALEs), a dCas protein, and/or a homeodomain.

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22. The rAAV of any one of claims 19-21, wherein:

~~—(i)—the at least one DNA binding domain binds to a nucleic acid sequence set forth in any one of SEQ ID NOs: 5-7; and/or~~

~~—(ii)—the at least one DNA binding domain is a zinc finger protein comprising a recognition helix encoded by a nucleic acid having a sequence set forth in any one of SEQ ID NOs: 11-16, 23-28, or 35-40.~~

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23. The rAAV of claim 22, wherein:

(a) the at least one DNA binding domain is a zinc finger protein comprising a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 11, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 12, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 13, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 14, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 15, and/or a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 16; or

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(b) the at least one DNA binding domain is a zinc finger protein comprising a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 23, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 24, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 25, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 26, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 27, and/or a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 28; or

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(c) the at least one DNA binding domain is a zinc finger protein comprising a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 35, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 36, a recognition helix encoded by a

nucleic acid comprising SEQ ID NO: 37, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 38, a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 39, and/or a recognition helix encoded by a nucleic acid comprising SEQ ID NO: 40.

5 24. The rAAV of any one of claims 19-23, wherein the at least one DNA binding domain is a zinc finger protein comprising the sequence set forth in any one of SEQ ID NOs: 17-22, 29-34, or 41-46.

25. The rAAV of claim 24, wherein:

10 (a) the at least one DNA binding domain is a zinc finger protein comprising a recognition helix comprising SEQ ID NO: 17, a recognition helix comprising SEQ ID NO: 18, a recognition helix comprising SEQ ID NO: 19, a recognition helix comprising SEQ ID NO: 20, a recognition helix comprising SEQ ID NO: 21, and/or a recognition helix comprising SEQ ID NO: 22; or

15 (b) the at least one DNA binding domain is a zinc finger protein comprising a recognition helix comprising SEQ ID NO: 29, a recognition helix comprising SEQ ID NO: 30, a recognition helix comprising SEQ ID NO: 31, a recognition helix comprising SEQ ID NO: 32, a recognition helix comprising SEQ ID NO: 33, and/or a recognition helix comprising SEQ ID NO: 34; or

20 (c) the at least one DNA binding domain is a zinc finger protein comprising a recognition helix comprising SEQ ID NO: 41, a recognition helix comprising SEQ ID NO: 42, a recognition helix comprising SEQ ID NO: 43, a recognition helix comprising SEQ ID NO: 44, a recognition helix comprising SEQ ID NO: 45, and/or a recognition helix comprising SEQ ID NO: 46.

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26. The rAAV of any one of claims 19-25, wherein the at least one DNA binding domain is a dCas protein, optionally a dCas9 protein, and wherein the rAAV further comprises at least one guide nucleic acid, optionally wherein the guide nucleic acid comprises a spacer sequence that targets *SCN1A*.

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27. The rAAV of claim 26, wherein the guide nucleic acid comprises a spacer sequence having a nucleotide sequence of any one of SEQ ID NO: 85, 86, 89, 90, 93, or 94.

28. The rAAV of claim 26 or 27, wherein the guide nucleic acid comprises a nucleotide sequence of any one of SEQ ID NOs: 83-94.

29. The rAAV of any one of claims 19-28, wherein the at least one transcriptional regulator domain is a transactivator derived from VRP, Rta, p65, Hsfl or any combination thereof.

30. The rAAV of any one of claims 19-29, wherein the transgene encoding the at least one transcriptional regulator transgene-domain comprises the sequence set forth in SEQ ID NO: 47 and/or the at least one transcriptional regulator domain is encoded by the amino acid sequence set forth in SEQ ID NO: 48.

31. The rAAV of any one of claims 19-30, wherein the at least one DNA binding domain is fused to the at least one transcriptional regulator domain by a linker domain, optionally wherein the linker domain is (i) a flexible linker, optionally comprised of glycines, or (ii) a cleavable linker.

32. The rAAV of any one of claims 19-31, wherein:

the transgene encodes 1 DNA binding domain, 2 DNA binding domains, 3 DNA binding domains, 4 DNA binding domains, 5 DNA binding domains, 6 DNA binding domains, 7 DNA binding domains, 8 DNA binding domains, 9 DNA binding domains, or 10 DNA binding domains; and/or

the transgene encodes 1 transcriptional regulator domain, 2 transcriptional regulator domains, 3 transcriptional regulator domains, 4 transcriptional regulator domains, 5 transcriptional regulator domains, 6 transcriptional regulator domains, 7 transcriptional regulator domains, 8 transcriptional regulator domains, 9 transcriptional regulator domains, or 10 transcriptional regulator domains.

33. The rAAV of any one of claims 19-32, wherein the AAV capsid serotype is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAV9, AAV10, AAVrh10, ~~or~~ and AAV.PHPB.

34. The rAAV of any one of claims 19-33, wherein the nucleic acid comprises an AAV2 ITR, optionally wherein the ITR is a Δ TR and/or a mTR.

35. The rAAV of any one of claims 19-34, wherein the transgene is operably linked to a promoter, optionally wherein the promoter is a tissue-specific promoter, further optionally wherein the tissue-specific promoter is a neuronal promoter, such as SST, NPY, Phosphate-activated glutaminase (PAG), Vesicular glutamate transporter-1 (VGLUT1), Glutamic acid decarboxylase 65 and 57 (GAD65, GAD67), Synapsin I, α-CamKII, Dock10, Prox1, Parvalbumin (PV), Somatostatin (SST), Cholecystokinin (CCK), Calretinin (CR), or Neuropeptide Y (NPY).

36. ~~An~~The isolated nucleic acid of any one of claims 1-18 or the rAAV of any one of claims 19-35 for use in a method of increasing expression of ~~a target~~an *SCN1A* gene, ~~the method comprising administering wherein~~ the isolated nucleic acid or the rAAV is to be administered to a cell or subject comprising the *SCN1A*target gene, ~~optionally wherein the target gene is *SCN1A*.~~

37. The isolated nucleic acid or the rAAV of claim 36, wherein the subject is haploinsufficient for the *SCN1A*target gene.

38. The isolated nucleic acid or the rAAV of any one of claims 36-37, wherein the cell is a neuron, optionally a GABAergic neuron.

39. ~~The~~An isolated nucleic acid of any one of claims 1-18 or the rAAV of any one of claims 19-35 for use in a method of treating Dravet syndrome in a subject expressing ~~a target~~an *SCN1A* gene, ~~the method comprising administering wherein~~ the isolated nucleic acid or the rAAV is to be administered to the subject, optionally wherein expression of the *SCN1A*target gene in the subject is decreased compared to a normal subject, ~~optionally wherein the target gene is *SCN1A*.~~

40. The isolated nucleic acid or the rAAV of claim 39, wherein the subject is or is suspected of being haploinsufficient in *SCN1A*target gene expression with respect to a normal subject and/or the subject has or is suspected of having a condition caused by haploinsufficient expression of the *SCN1A*target gene.

41. The isolated nucleic acid or the rAAV of any one of claims 36-40, wherein the isolated nucleic acid or the rAAV is administered by intravenous injection, intramuscular injection, inhalation, subcutaneous injection, and/or intracranial injection.

5 42. The isolated nucleic acid or the rAAV of any one of claims 36-41, wherein
administration of the isolated nucleic acid or the rAAV results in SCN1A~~target~~ gene
expression that is increased by at least 2-fold, at least 10-fold, at least 20-fold, at least 30-fold,
at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-
fold, or at least 100-fold relative to expression of the transgene in the subject prior to
10 administration.

43. A composition comprising the isolated nucleic acid of any of claims 1-18 or the rAAV
of any one of claims 19-35, optionally further comprising a pharmaceutically acceptable
carrier.

15 44. A kit comprising:
a container housing an isolated nucleic acid of any one of claims 1-18 or the rAAV of
any one of claims 19-35, optionally wherein the kit further comprises a container housing a
pharmaceutically acceptable carrier, further optionally wherein the isolated nucleic acid of the
20 rAAV and the pharmaceutically acceptable carrier are housed in the same container, and
optionally wherein the container is a syringe.

45. A host cell comprising the isolated nucleic acid of any one of claims 1-18 or the rAAV
of any one of claims 19-35.

25 46. The host cell of claim 45, wherein the host cell is a eukaryotic cell, a mammalian cell,
or a human cell, optionally a neuron, further optionally a GABAergic neuron.