

Claims

1. An isolated polynucleotide that specifically binds a *Grik2* mRNA comprising a stem-loop region comprising a 5' arm (5p), a loop region, and a 3' arm (3p), wherein the stem-loop region comprises a guide strand sequence and a passenger strand sequence, and the guide strand sequence and passenger strand sequence comprises:
 - (a) a uracil(U)-adenine(A) base pair or a U-guanine(G) base pair at the 5' end of the guide strand;
 - (b) a cytosine(C)-G pair at the 5' end of the passenger strand;
 - (c) a U at the 5' end of the guide strand sequence;
 - (d) a mismatch in a seed region between the guide strand and passenger strand sequences;and/or
 - (e) a C-G base pair or U-A base pair to replace a U-G wobble at a junction of the stem region and the loop region of the polynucleotide.
2. The polynucleotide of claim 1, wherein:
 - (i) (a) and (c) improve guide strand sequence loading into an RNA-induced silencing complex (RISC) protein;
 - (ii) (b) impairs passenger strand sequence loading into a RISC protein;
 - (iii) (d) promotes decoupling of the passenger strand sequence from the guide strand sequence during RISC loading;
 - (iv) (e) improves cleavage of the loop region from the stem region by Dicer; and/or
 - (v) the seed region of the guide strand sequence comprises nucleotides 2 through 7 of the guide strand sequence.
3. The polynucleotide of claim 1 or 2, wherein:
 - (a) the stem-loop region is a polynucleotide having at least 85% sequence identity to the nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-15, 47-62, 98-108, 134-138, 226-229, and 238-241;
 - (b) the guide strand sequence has a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 17-30, 64-79, 110-120, 140-144, 230-233, and 242-245; and/or
 - (c) the passenger strand sequence has a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 32-45, 81-96, 122-132, 146-150, 234-237, and 246-249.
4. The polynucleotide of any one of claims 1-3, further comprising a second stem-loop region, wherein the stem-loop region is a polynucleotide having at least 85% sequence identity to the nucleic acid

sequence of SEQ ID NO: 4 or 135, and the second stem-loop region is a polynucleotide having at least 85% sequence identity to the nucleic acid sequence of SEQ ID NO: 4 or 135.

5. The polynucleotide of any one of claims 1-4, wherein the polynucleotide is or comprises an antisense oligonucleotide (ASO), a short interfering RNA (siRNA), a short hairpin RNA (shRNA), a microRNA (miRNA), or a short hairpin-adapted miRNA (shmiRNA), optionally wherein the polynucleotide is 19 nucleotides, 20 nucleotides, or 21 nucleotides in length.

6. The polynucleotide of any one of claims 1-5, wherein the *Grik2* mRNA is encoded by a nucleic acid sequence of SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, or SEQ ID NO: 174.

7. The polynucleotide of any one of claims 1-6, wherein the polynucleotide is capable of reducing a level of GluK2 protein in a cell, wherein the polynucleotide reduces a level of GluK2 protein in the cell by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75%.

8. The polynucleotide of claim 7, wherein the cell is:
(a) a human cell; and/or
(b) a neuron, optionally a hippocampal neuron, further optionally a dentate granule cell (DGC) or a glutamatergic pyramidal neuron.

9. A vector comprising the polynucleotide of any one of claims 1-8.

10. The vector of claim 9, wherein the vector is:
(a) replication-defective;
(b) a mammalian, insect, or bacterial vector;
(c) an expression vector; and/or
(d) a viral vector, optionally selected from the group consisting of an adeno-associated virus (AAV), retrovirus, adenovirus, parvovirus, coronavirus, negative strand RNA viruses, orthomyxovirus, rhabdovirus, paramyxovirus, positive strand RNA viruses, picornavirus, alphavirus, a double stranded DNA virus, herpesvirus, Epstein-Barr virus, cytomegalovirus, fowlpox virus, and canarypox virus.

11. The vector of claim 10 (d), wherein the AAV vector is optionally an AAV5, AAV9, or AAVrh10 vector.

12. An expression cassette comprising a polynucleotide comprising a stem-loop sequence having at least 85% sequence identity to a nucleic acid sequence of any one of SEQ ID NOs: 1-15, 46-62, 97-108, 133-138, 226-229, 238-241, and 256-261, such as a polynucleotide comprising at least 85% sequence identity to a nucleic acid sequence of any one of SEQ ID NOs: 4, 135, and 256-261.
13. The expression cassette of claim 12, wherein the expression cassette comprises:
- (a) a 5' flanking region, wherein the 5' flanking region optionally comprises:
 - (i) a polynucleotide having at least 85% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 217, 220, or 223; and/or
 - (ii) a 5' spacer sequence and a 5' flanking sequence;
 - (b) a 3' flanking region, wherein the 3' flanking region optionally comprises:
 - (i) a polynucleotide having at least 85% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 218, 221, or 224; and/or
 - (ii) a 3' spacer sequence and a 3' flanking sequence;
 - (c) a loop region, wherein the loop region optionally comprises:
 - (i) a microRNA loop sequence that is a E-miR-30, miR-218-1, or E-miR-124-3 sequence; and/or
 - (ii) a polynucleotide having at least 85% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 219, 222, or 225; and/or
 - (d) a promoter, wherein the promoter comprises a Synapsin (hSyn) promoter or Calcium/Calmodulin Dependent Protein Kinase II (CaMKII) promoter.
14. An expression cassette comprising, from 5' to 3':
- (a) a promoter sequence;
 - (b) a first polynucleotide comprising a stem-loop sequence having at least 85% sequence identity to a nucleic acid sequence of any one of SEQ ID NOs: 1-19, 34-62, 97-108, 133-147, 226-229, and 238-241; and
 - (c) a polynucleotide comprising a stem-loop sequence having at least 85% sequence identity to a nucleic acid sequence of any one of SEQ ID NOs: 1-19, 34-62, 97-108, 133-147, 226-229, and 238-241.
15. The expression cassette of claim 14, wherein each of the first polynucleotide and the second polynucleotide independently comprise:
- (a) a passenger sequence which is complementary or substantially complementary to a guide sequence, wherein the passenger sequence is located 5' or 3' relative to the guide sequence;

- (b) a 5' flanking region located 5' relative to a guide sequence, optionally comprising a polynucleotide having at least 85% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 217, 220, or 223;
 - (c) a 3' flanking region located 3' relative to a guide sequence, optionally comprising a polynucleotide having at least 85% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 218, 221, or 224; and/or
 - (d) a loop region located between a guide sequence and a passenger sequence, wherein the loop region comprises a microRNA loop sequence, optionally a E-miR-30, miR-218-1, or E-miR-124-3 sequence, further optionally having at least 85% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 219, 222, or 225.
16. The expression cassette of claim 14 or 15, wherein:
- (a) the promoter is selected from the group consisting of an hSyn promoter or CaMKII promoter;
 - (b) the expression cassette comprises a 5'-inverted terminal repeat (ITR) sequence on the 5' end of said expression cassette and a 3'-ITR sequence on the 3' end of said expression cassette, wherein optionally:
 - (i) the 5'-ITR and 3' ITR sequences are AAV2 5'-ITR and 3' ITR sequences; and/or
 - (ii) the 5'-ITR sequence comprises a polynucleotide having at least 85% sequence identity to the nucleic acid sequence of SEQ ID NOs: 208 or 209; and/or
 - (iii) the 3'-ITR sequence comprises a polynucleotide having at least 85% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 210-212;
 - (c) the expression cassette further comprises an enhancer sequence, optionally wherein the enhancer sequence comprises a polynucleotide having at least 85% sequence identity to the nucleic acid sequence of SEQ ID NO: 207;
 - (d) the expression cassette further comprises an intron sequence, optionally wherein the intron sequence comprises a polynucleotide having at least 85% sequence identity to the nucleic acid sequence of SEQ ID NO: 205 or SEQ ID NO: 206;
 - (e) the expression cassette further comprises one or more polyadenylation signal sequences, wherein optionally:
 - (i) the one or more polyadenylation signal sequences is a rabbit beta-globin (RBG) polyadenylation signal sequence, further optionally comprising a polynucleotide having at least 85% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 213-215; and/or
 - (ii) the one or more polyadenylation signal sequences is a bovine growth hormone (BGH) polyadenylation signal sequence, further optionally comprising a polynucleotide having at least 85% sequence identity to the nucleic acid sequence of SEQ ID NO: 216;
 - (f) the expression cassette is incorporated into the vector of any one of claims 9-11; and/or

(g) the expression cassette comprises at least 80% sequence identity to the sequence of SEQ ID NO: 256, optionally at least 85%, 90%, 95% 97%, sequence identity to the sequence of SEQ ID NO: 256.

17. A method of inhibiting *Grik2* expression in a cell comprising contacting the cell with at least one polynucleotide of any one of claims 1-8, the vector of any one of claims 9-11, or the expression cassette of any one of claims 12-16, wherein optionally:

- (a) the polynucleotide specifically hybridizes to a *Grik2* mRNA and inhibits or reduces the expression of *Grik2* in the cell;
- (b) the method reduces a level of GluK2 protein in the cell, wherein the level of GluK2 protein in the cell is reduced at least 10%, at least 10%, at least at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75%;
- (c) the cell is a human cell; and/or
- (d) the cell is a neuron, optionally a hippocampal neuron, further optionally wherein the hippocampal neuron is a glutamatergic pyramidal neuron or a DGC, wherein the DGC optionally comprises an aberrant recurrent mossy fiber axon.

18. Use of a composition comprising the polynucleotide of any one of claims 1-8, the vector of any one of claims 9-11, or the expression cassette of any one of claims 12-16 in the manufacture of a pharmaceutical composition for treating or ameliorating an epilepsy, wherein the epilepsy is optionally a temporal lobe epilepsy (TLE), chronic epilepsy, and/or a refractory epilepsy, further optionally wherein the TLE is a lateral TLE or a mesial TLE.

19. A pharmaceutical composition comprising the polynucleotide of any one of claims 1-8, the vector of any one of claims 9-11, or the expression cassette of any one of claims 12-16, and a pharmaceutically acceptable carrier, diluent, or excipient.

20. A kit comprising the pharmaceutical composition of claim 19, and a package insert, wherein the package insert comprises instructions for use of the pharmaceutical composition of claim 18.