

CLAIMS

What is claimed is:

1. An RNA-targeting nucleic acid molecule comprising an engineered snRNA (esnRNA), wherein the esnRNA system comprises:
 - (i) an engineered stem loop (eSL) comprising one or more nucleic acid sequences selected from SEQ ID NOs: 1 – 11, SEQ ID NOs: 146 – 148, SEQ ID NO: 163, or SEQ ID NOs: 186 – 205;
 - (ii) a 5' interaction stabilizing domain (5' ISD) comprising nucleotides complementary to the eSL, wherein the 5' ISD anneals to and/or hybridizes to the eSL; and
 - (iii) a targeting sequence that targets a target RNA of interest.
2. The RNA-targeting nucleic acid molecule of claim 1, wherein the target RNA of interest is pre-mRNA, mRNA or a microsatellite repeat RNA.
3. The RNA-targeting nucleic acid molecule of claim 2, wherein the microsatellite repeat RNA is selected from the group consisting of CUG, CAG, and GGGGCC+CCCCGG.
4. The RNA-targeting nucleic acid molecule of any one of claims 1-3, wherein the target RNA of interest is a sequence encoding DMD.
5. The RNA-targeting nucleic acid molecule of any one of claims 1-4, wherein the targeting sequence is selected from the group consisting of SEQ ID NO: 206 and SEQ ID NO: 207 or comprises one or more nucleic acid sequences set forth in SEQ ID NOs: 208 – 227.
6. The RNA-targeting nucleic acid molecule of any one of claims 1-5, wherein the esnRNA comprises two targeting sequences that target two RNAs of interest.
7. The RNA-targeting nucleic acid molecule of claim 6, wherein the two targeting sequences are a fusion sequence.
8. The RNA-targeting nucleic acid molecule of any one of claims 1-7, wherein the esnRNA comprises an Sm binding domain (SmBD) selected from the group consisting of a U1, a U2, a U4, and a U5 SmBD.
9. The RNA-targeting nucleic acid molecule of claim 8, wherein the SmBD comprises a nucleic acid sequence set forth in SEQ ID NOs: 31 – 38, or 164.

10. The RNA-targeting nucleic acid molecule of any one of claims 1-9, wherein the 5'ISD comprises a nucleotide sequence selected from the group consisting of ggagt, cctct, ggaggt, cctcct, agccag, ggaag, gaagaag, gttg, ccgaa, taaggag, gaag and ggctt.
11. The RNA targeting nucleic acid of any one of claims 1-10, wherein the esnRNA comprises a nucleic acid sequence of SEQ ID NOs: 65 – 119 or 179 – 185.
12. A vector comprising one or more esnRNA of any one of claims 1-11.
13. The vector of claim 12, wherein the vector is an AAV vector.
14. The AAV vector of claim 13, wherein:
 - (i) the snRNA is operably linked to a promoter;
 - (ii) the snRNA is operably linked to a U7 promoter or a U1 promoter;
 - (iii) the snRNA is operably linked to a downstream terminator (DT);
 - (iv) the snRNA is operably linked to a U7 downstream terminator or a U1 downstream terminator, or
 - (v) the vector comprises at least one, at least two, at least three, at least four, or at least five esnRNA.
15. The AAV vector of claim 14, wherein the at least one, at least two, at least three, at least four, or at least five esnRNA each target the same target RNA sequences, target two or more target RNA sequences, or each esnRNA is separated by a buffer sequence.
16. The AAV vector of claim 15, wherein the buffer sequence comprises a nucleic acid sequence set forth in any one of SEQ ID NOs: 24 – 30.
17. The AAV vector of claim 14 or 15, wherein the vector comprises a nucleic acid sequence set forth in any one of SEQ ID NOs: 123 – 143, 168 – 178, or 231 – 233.
18. Use of the RNA-targeting nucleic acid molecule of any one of claims 1-11 or the AAV vector of any one of claims 13-17 in the manufacture of a medicament for treating a disease or disorder in a subject.
19. The use according to claim 18, wherein the treating comprises targeting one or more target RNAs of interest and blocking, knocking down, editing, exon-skipping or splicing the one or more target RNAs, and/or comprises contacting the esnRNA with a cell comprising the one or more target RNAs.
20. The use according to claim 18 or 19, wherein:
 - (i) the disease or disorder is associated with a toxic repeat RNA sequence,
 - (ii) the toxic repeat RNA sequence is a CAG, CUG, GGCCCC, CCGGG, or GGCCCC+CCGGGG RNA repeat,

(iii) the disease or disorder is myotonic dystrophy (DM1) or Huntington's disease (HD),

(iv) the disease or disorder is Duchenne Muscular Dystrophy, or

(v) the medicament is to be administered via intravenous, intramuscular, subpial, intrathecal, intraparenchymal, intrathecal, intrastriatal, subcutaneous, intradermal, intraperitoneal, intratumoral, intravenous, intraocular, and/or parenteral administration.

21. The use according to any one of claims 18-20, wherein the RNA-targeting nucleic acid molecule or AAV vector targets an RNA sequence encoding dystrophin (DMD) and,

wherein the RNA sequence encoding DMD comprises an intronic or exonic sequence.

22. The use of claim 21, wherein the exonic sequence comprises exon 51, or a flanking region thereof, of DMD.