[0005] Current CRISPR/Cas RNA targeting systems typically use a single guide RNA and optionally an oligonucleotide of alternating 2' OMe RNA and DNA bases (PAMmer) to provide a simple and rapidly programmable system for targeting of specific RNA molecules in live cells. However, improvements and/or alternatives to these systems can help address issues relating to efficiency, specificity and/or off-target editing events. The present disclosure addresses these needs and provides related advantages.

SUMMARY OF THE DISCLOSURE

[0006] Accordingly, provided herein are fully encodable and highly specific CRISPR/Cas systems, compositions, and methods to achieve efficient and reversible manipulation and modulation of target RNA with simplicity, reliability and versatility.

[0007] In some examples, provided herein are recombinant expression systems for CRISPR/Cas-directed RNA editing of a target RNA comprising, consisting of, or consisting essentially of: (A) a nucleic acid sequence encoding a CRISPR/Cas RNA editing fusion protein comprising a nuclease-dead CRISPR associated endonuclease (dCas) fused to a catalytically active deaminase domain of Adenosine Deaminase acting on RNA (ADAR); and (B) a nucleic acid sequence encoding an extended single guide RNA (esgRNA) comprising: (i) a short extension sequence of homology to the target RNA comprising a mismatch for a target adenosine, and (ii) a dCas scaffold binding sequence. In one aspect, the present disclosure refers to a recombinant expression system for CRISPR/Cas-directed RNA editing of a target RNA comprising: (A) a nucleic acid sequence encoding a CRISPR/Cas RNA editing fusion protein comprising a nuclease-dead CRISPR associated endonuclease (dCas) fused to a catalytically active deaminase domain of Adenosine Deaminase acting on RNA 2 (ADAR2); and (B) a nucleic acid sequence encoding an extended single guide RNA (esgRNA) comprising: (i) a short extension sequence of homology to the target RNA comprising a mismatch for a target adenosine, and (ii) a dCas9 scaffold binding sequence. In some examples, said expression system expresses a dCas-ADAR nucleoprotein complex capable of CRISPR/Cas RNA-RNA base-specific Adenosine to Inosine (A – I) editing of the target sequence.

[0008] In some examples of the recombinant expression systems, the esgRNA further comprises (iii) a spacer sequence comprising a region of homology to the target RNA.

[0009] In some examples of the recombinant expression systems, (A) and (B) are comprised within the same vector or comprised within different vectors. In some examples of the recombinant expression systems, the vector is a viral vector. In some examples of the recombinant expression systems, the viral vector is an adeno-associated viral vector (AAV), lentiviral vector, or an adenoviral vector.

[0014] In some examples of the recombinant expression systems, the esgRNA further comprises a RNA polymerase III promoter sequence. In some examples of the recombinant expression systems, the RNA polymerase III promoter sequence is a U6 promoter sequence.

[0015] In some examples of the recombinant expression systems, the esgRNA comprises a linker sequence between the spacer sequence and the scaffold sequence.

[0016] In some examples of the recombinant expression systems, the sequences of the esgRNA (i), (ii), and (iii) are situated 3' to 5' in the esgRNA.

[0017] In some examples of the recombinant expression systems, the expression system further comprises a nucleic acid encoding a PAM sequence.

[0018] In some examples, provided herein are vectors comprising, consisting of, or consisting essentially of a nucleic acid encoding an extended single guide RNA (esgRNA) comprising (i) a short extension sequence of homology to a target RNA comprising a mismatch for a target adenosine, (ii) a dCas scaffold binding sequence, and (iii) a sequence complementary to the target sequence (spacer sequence), wherein (i), (ii) and (iii) are situated 3' to 5' in the esgRNA. In one example, the present disclosure refers to a vector comprising a nucleic acid encoding an extended single guide RNA (esgRNA) comprising (i) a short extension sequence of homology to a target RNA comprising a mismatch for a target adenosine, (ii) a dCas scaffold binding sequence, and (iii) a sequence complementary to the target sequence (spacer sequence), wherein (i), (ii) and (iii) are situated 3' to 5' in the esgRNA.

[0019] In some examples of the vectors, the vector is a viral vector. In some examples of the vectors, the viral vector is an adeno-associated viral vector (AAV), lentiviral vector, or an adenoviral vector. In some examples of the vectors, the vectors further comprise an expression control element.

[0020] In one example, the present disclosure refers to a viral particle comprising any one of the vectors disclosed herein. In some examples, provided herein are viral particles comprising a vector comprising, consisting of, or consisting essentially of a nucleic acid encoding an extended single guide RNA (esgRNA) comprising (i) a short extension sequence of homology to a target RNA comprising a mismatch for a target adenosine, (ii) a dCas scaffold binding sequence, and (iii) a sequence complementary to the target sequence (spacer sequence), wherein (i), (ii) and (iii) are situated 3' to 5' in the esgRNA. In some examples, provided herein are viral particles comprising one or more vectors comprising (A) a nucleic acid sequence encoding a CRISPR/Cas RNA editing fusion protein comprising a nuclease-dead CRISPR associated

endonuclease (dCas) fused to a catalytically active deaminase domain of Adenosine Deaminase acting on RNA (ADAR); and (B) a nucleic acid sequence encoding an extended single guide RNA (esgRNA) comprising: (i) a short extension sequence of homology to the target RNA comprising a mismatch for a target adenosine, and (ii) a dCas scaffold binding sequence.

[0021] In one aspect, the present disclosure refers to a cell comprising any one of the recombinant expression systems disclosed herein. In some examples, provided herein are cells comprising recombinant expression systems, viral particles, and/or vectors comprising, consisting of, or consisting essentially of a nucleic acid encoding an extended single guide RNA (esgRNA) comprising (i) a short extension sequence of homology to a target RNA comprising a mismatch for a target adenosine, (ii) a dCas scaffold binding sequence, and (iii) a sequence complementary to the target sequence (spacer sequence), wherein (i), (ii) and (iii) are situated 3' to 5' in the esgRNA. In some examples, provided herein are cells comprising one or more viral particles, recombinant expression systems, and/or vectors comprising (A) a nucleic acid sequence encoding a CRISPR/Cas RNA editing fusion protein comprising a nuclease-dead CRISPR associated endonuclease (dCas) fused to a catalytically active deaminase domain of Adenosine Deaminase acting on RNA (ADAR); and (B) a nucleic acid sequence encoding an extended single guide RNA (esgRNA) comprising: (i) a short extension sequence of homology to the target RNA comprising a mismatch for a target adenosine, and (ii) a dCas scaffold binding sequence.

[0021a] In one example, the present disclosure refers to an esgRNA comprising: (i) a short extension sequence of homology to a target RNA comprising a mismatch for a target adenosine, and (ii) a dCas scaffold binding sequence. In another example, the present disclosure refers to a CRISPR/Cas RNA editing fusion protein comprising a nuclease-dead CRISPR associated endonuclease (dCas) fused to a catalytically active deaminase domain of ADAR.

[0022] In one aspect, the present disclosure refers to a method of selective RNA editing comprising administering any one of the recombinant expression systems as disclosed herein to a cell, wherein the method is *in vitro*. Also provided herein are methods of selective RNA editing comprising, consisting of, or consisting essentially of administering any one of the recombinant expression systems, viral particles, and/or vectors comprising, consisting of, or consisting essentially of a nucleic acid encoding an extended single guide RNA (esgRNA) comprising (i) a short extension sequence of homology to a target RNA comprising a mismatch for a target adenosine, (ii) a dCas scaffold binding sequence, and (iii) a sequence complementary to the target sequence (spacer sequence), wherein (i), (ii) and (iii) are situated 3' to 5' in the esgRNA to a cell. In some examples, the methods further comprise administering an antisense synthetic oligonucleotide compound comprising alternating 2'OMe RNA and DNA bases (PAMmer). In some examples, the method is *in vitro* or *in vivo*. In some examples, provided herein are methods of selective RNA editing comprising, consisting of, or consisting

essentially of administering any one of the recombinant expression systems, viral particles, and/or vectors comprising, consisting of, or consisting essentially of (A) a nucleic acid sequence encoding a CRISPR/Cas RNA editing fusion protein comprising a nuclease-dead CRISPR associated endonuclease (dCas) fused to a catalytically active deaminase domain of Adenosine Deaminase acting on RNA (ADAR); and (B) a nucleic acid sequence encoding an extended single guide RNA (esgRNA) comprising: (i) a short extension sequence of homology to the target RNA comprising a mismatch for a target adenosine, and (ii) a dCas scaffold binding sequence.

[0023] In one aspect, the present disclosure refers to a method of characterizing the effects of directed cellular RNA editing on processing and dynamics comprising administering any one of the recombinant expression systems as disclosed herein to a sample and determining its effects. Also provided herein are methods of characterizing the effects of directed cellular RNA editing on processing and dynamics comprising administering any one of the recombinant expression systems, viral particles, and/or vectors comprising, consisting of, or consisting essentially of a nucleic acid encoding an extended single guide RNA (esgRNA) comprising (i) a short extension sequence of homology to a target RNA comprising a mismatch for a target adenosine, (ii) a dCas scaffold binding sequence, and (iii) a sequence complementary to the target sequence (spacer sequence), wherein (i), (ii) and (iii) are situated 3' to 5' in the esgRNA to a sample and determining its effects. In some examples, the sample is derived from a subject. In some examples, the method is *in vitro* or *in vivo*. In some examples, provided herein are methods of characterizing the effects of directed cellular RNA editing on processing and dynamics comprising administering any one of the recombinant expression systems, viral particles, and/or vectors comprising, consisting of, or consisting essentially of (A) a nucleic acid sequence encoding a CRISPR/Cas RNA editing fusion protein comprising a nuclease-dead CRISPR associated endonuclease (dCas) fused to a catalytically active deaminase domain of Adenosine Deaminase acting on RNA (ADAR); and (B) a nucleic acid sequence encoding an extended single guide RNA (esgRNA) comprising: (i) a short extension sequence of homology to the target RNA comprising a mismatch for a target adenosine, and (ii) a dCas scaffold binding sequence to a sample and determining its effects.

[0024] In one aspect, the present disclosure refers to use of any one of the recombinant expression systems as disclosed herein, or the cell as disclosed herein, in the manufacture of a medicament for treating a disease selected from the group consisting of Hurler's syndrome, Cystic fibrosis, muscular dystrophy, spinal cord injury, hearing loss (through noise overexposure or ototoxicity), multiple sclerosis, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Parkinson's disease, over-rapid benzodiazepine withdrawal, and Huntington's disease in a subject, wherein the medicament is to be administered to a subject or a sample isolated from a subject. In other examples, provided herein are methods of treating a disease or condition in a subject comprising administering any one of the recombinant expression systems, viral particles, and/or vectors comprising, consisting of, or consisting essentially of a nucleic acid

encoding an extended single guide RNA (esgRNA) comprising (i) a short extension sequence of homology to a target RNA comprising a mismatch for a target adenosine, (ii) a dCas scaffold binding sequence, and (iii) a sequence complementary to the target sequence (spacer sequence), wherein (i), (ii) and (iii) are situated 3' to 5' in the esgRNA to a subject or a sample isolated from a subject. In some examples, provided herein are methods of treating a disease or condition in a subject comprising administering any one of the recombinant expression systems, viral particles, and/or vectors comprising, consisting of, or consisting essentially of (A) a nucleic acid sequence encoding a CRISPR/Cas RNA editing fusion protein comprising a nuclease-dead CRISPR associated endonuclease (dCas) fused to a catalytically active deaminase domain of Adenosine Deaminase acting on RNA (ADAR); and (B) a nucleic acid sequence encoding an extended single guide RNA (esgRNA) comprising: (i) a short extension sequence of homology to the target RNA comprising a mismatch for a target adenosine, and (ii) a dCas scaffold binding sequence to a subject or a sample isolated from a subject.

[0025] In some examples, the methods further correcting a G to A mutation in a target RNA. In some examples, the disease is selected from the group of Hurler's syndrome, Cystic fibrosis, Duchenne muscular dystrophy, spinal cord injury, stroke, traumatic brain injury, hearing loss (through noise overexposure or ototoxicity), multiple sclerosis, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Parkinson's disease, alcoholism, alcohol withdrawal, over-rapid benzodiazepine withdrawal, and Huntington's disease.

[0026] In one aspect, the present disclosure refers to a kit comprising: (A) one or more of: (i) a recombinant expression system as disclosed herein; (ii) a vector comprising a nucleic acid encoding an esgRNA comprising (a) a short extension sequence of homology to a target RNA comprising a mismatch for a target adenosine, and (b) a dCas scaffold binding sequence; (iii) a vector comprising a nucleic acid encoding a dCas fused to a catalytically active deaminase domain of ADAR; (iv) a viral particle comprising the recombinant expression system as disclosed herein; (v) a cell comprising the recombinant expression system as disclosed herein; (vi) an esgRNA comprising (a) a short extension sequence of homology to a target RNA comprising a mismatch for a target adenosine, and (b) a dCas scaffold binding sequence; and/or (vii) a CRISPR/Cas RNA editing fusion protein comprising a dCas fused to a catalytically active deaminase domain of ADAR; and (B) instructions for use. In other examples, provided herein are kits comprising, consisting of, or consisting of one or more of: recombinant expression systems, viral particles, and/or vectors comprising, consisting of, or consisting essentially of (A) a nucleic acid sequence encoding a CRISPR/Cas RNA editing fusion protein comprising a nuclease-dead CRISPR associated endonuclease (dCas) fused to a catalytically active deaminase domain of Adenosine Deaminase acting on RNA (ADAR); and (B) a nucleic acid sequence encoding an extended single guide RNA (esgRNA) comprising: (i) a short extension sequence of homology to the target RNA comprising a mismatch for a target adenosine, and (ii) a dCas scaffold binding sequence and instructions for use. In some examples, the instructions are for use according to any one of the methods described herein.

WHAT IS CLAIMED IS:

1. A recombinant expression system for CRISPR/Cas-directed RNA editing of a target RNA comprising:

(A) a nucleic acid sequence encoding a CRISPR/Cas RNA editing fusion protein comprising a nuclease-dead CRISPR associated endonuclease (dCas) fused to a catalytically active deaminase domain of Adenosine Deaminase acting on RNA 2 (ADAR2); and

(B) a nucleic acid sequence encoding an extended single guide RNA (esgRNA) comprising: (i) a short extension sequence of homology to the target RNA comprising a mismatch for a target adenosine, and (ii) a dCas9 scaffold binding sequence.

2. The recombinant expression system of claim 1, wherein the esgRNA further comprises (iii) a spacer sequence comprising a region of homology to the target RNA.

3. The recombinant expression system of claim 1, wherein (A) and (B) are comprised within the same vector or comprised within different vectors.

4. The recombinant expression system of claim 1, wherein the catalytically active deaminase domain of ADAR2 is (1) a wildtype catalytically active deaminase domain of human ADAR2 or (2) a mutant human catalytically active deaminase domain of ADAR2 with increased catalytic activity compared to the wildtype human ADAR2.

5. The recombinant expression system of claim 4, wherein the mutant human catalytically active deaminase domain of ADAR2 comprises a E488Q mutation.

6. The recombinant expression system of claim 1, wherein the dCas9 N-terminal domain is fused to the C-terminus of the catalytically active deaminase domain of ADAR2.

7. The recombinant expression system of any one of the preceding claims, wherein the dCas9 is fused to the catalytically active deaminase domain of ADAR2 via a linker.

8. The recombinant expression system of claim 7, wherein the linker is a semi-flexible XTEN peptide linker.

9. The recombinant expression system of any one of the previous claims, wherein the short extension sequence of the esgRNA is a 3' extension sequence.

10. The recombinant expression system of any one of the previous claims, wherein the short extension sequence of the esgRNA comprises a region of homology capable of near-perfect RNA-RNA base pairing with the target sequence.

11. The recombinant expression system of any one of the preceding claims, wherein the short extension sequence of the esgRNA further comprises a second mismatch for an adenosine within the target RNA.

12. The recombinant expression system of claim 11, wherein the short extension sequence of the esgRNA further comprises a third mismatch for an adenosine within the target RNA and optionally a fourth mismatch for an adenosine within the target RNA.

13. The recombinant expression system of any one of the previous claims, wherein the short extension sequence of the esgRNA is 15 nucleotides to 60 nucleotides in length.

14. The recombinant expression system of any one of the previous claims, wherein the esgRNA further comprises a marker sequence.

15. The recombinant expression system of any one of the previous claims, wherein the esgRNA further comprises a RNA polymerase III promoter sequence.

16. The recombinant expression system of claim 15, wherein the RNA polymerase III promoter sequence is a U6 promoter sequence.

17. The recombinant expression system of any one of the previous claims, wherein the esgRNA comprises a linker sequence between a spacer sequence and a scaffold sequence.

18. The recombinant expression system of claim 2, wherein the sequences of the esgRNA (i), (ii), and (iii) are situated 3' to 5' in the esgRNA.

19. The recombinant expression system of any one of the previous claims, further comprising a nucleic acid encoding a PAM sequence or complement thereof.

20. The recombinant expression system of claim 3, wherein the vector is a viral vector.

21. The recombinant expression system of claim 20, wherein the viral vector is an adeno-associated viral vector (AAV), lentiviral vector, or an adenoviral vector.

22. A cell comprising any one of the recombinant expression systems of claims 1 to 21.

23. A method of selective RNA editing comprising administering any one of the recombinant expression systems of claims 1 to 21 to a cell, wherein the method is *in vitro*.

24. The method of claim 23, further comprising administering an antisense synthetic oligonucleotide compound comprising alternating 2'OMe RNA and DNA bases (PAMmer).

25. A method of characterizing the effects of directed cellular RNA editing on processing and dynamics comprising administering any one of the recombinant expression systems of claims 1 to 21 to a sample and determining its effects.

26. The method of claim 25, wherein the sample is derived from a subject.

27. Use of any one of the recombinant expression systems of claims 1 to 21, or the cell of claim 22, in the manufacture of a medicament for treating a disease selected from the group consisting of Hurler's syndrome, Cystic fibrosis, muscular dystrophy, spinal cord injury, hearing loss (through noise overexposure or ototoxicity), multiple sclerosis, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Parkinson's disease, over-rapid benzodiazepine withdrawal, and Huntington's disease in a subject, wherein the medicament is to be administered to a subject or a sample isolated from a subject.

28. The use of claim 27, further comprising correction of a G to A mutation in a target RNA.

29. A kit comprising:

(A) one or more of:

(i) a recombinant expression system according to any one of claims 1 to 21;

(ii) a vector comprising a nucleic acid encoding an esgRNA comprising

(a) a short extension sequence of homology to a target RNA comprising a mismatch for a target adenosine, and (b) a dCas scaffold binding sequence;

(iii) a vector comprising a nucleic acid encoding a dCas fused to a catalytically active deaminase domain of ADAR;

(iv) a viral particle comprising the recombinant expression system according to any one of claims 1 to 21;

(v) a cell comprising the recombinant expression system according to any one of claims 1 to 21;

(vi) an esgRNA comprising (a) a short extension sequence of homology to a target RNA comprising a mismatch for a target adenosine, and (b) a dCas scaffold binding sequence; and/or

(vii) a CRISPR/Cas RNA editing fusion protein comprising a dCas fused to a catalytically active deaminase domain of ADAR; and

(B) instructions for use.

30. The kit of claim 29, wherein the instructions for use are according to a method comprising administering the one or more of part (A) to a cell, a sample isolated from a subject, or to a subject.