[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 (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 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.

[0010] In some examples of the recombinant expression systems, the ADAR is selected from the group consisting of ADAR1, ADAR2, and ADAR3. In some examples, the catalytically active deaminase domain of ADAR is the catalytically active deaminase domain of ADAR2. In some examples of the recombinant expression systems, 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. In some examples of the recombinant expression systems, the mutant human catalytically active deaminase domain of ADAR2 comprises a E488Q mutation.

[0011] In some examples of the recombinant expression systems, the dCas is nuclease-dead Cas9 (dCas9). In some examples of the recombinant expression systems, the dCas9 N-terminal domain is fused to the C-terminus of the catalytically active deaminase domain of ADAR. In some examples of the recombinant expression systems, the dCas is fused to the catalytically active deaminase domain of ADAR via a linker. In some examples of the recombinant expression systems, the linker is a semi-flexible XTEN peptide linker. In some examples, the linker is a GSGS linker.

[0012] In some examples of the recombinant expression systems, the short extension sequence of the esgRNA is a 3' extension sequence. In some examples of the recombinant expression systems, the short extension sequence of the esgRNA comprises a region of homology capable of near-perfect RNA-RNA base pairing with the target sequence. In some examples of the recombinant expression systems, the short extension sequence of the esgRNA further comprises a second mismatch for an adenosine within the target RNA. In some examples of the recombinant expression systems, 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. In some examples of the recombinant expression systems, the short extension sequence of the esgRNA is about 15 nucleotides to about 60 nucleotides in length.

[0013] In some examples of the recombinant expression systems, the esgRNA further comprises a marker sequence.

[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 aspect, 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 aspect, 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, the vectors disclosed herein, or the viral particles 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 aspect, 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 aspect, 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, the vectors as disclosed herein, or the viral particle as disclosed herein to a cell. 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, the vectors as disclosed herein, the viral particle as disclosed herein, or the cell 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, the vectors as disclosed herein, the viral particle as disclosed herein, or the cell as disclosed herein, in the manufacture of a medicament for treating a disease or condition 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) recombinant expression system as disclosed herein; (ii) vector as disclosed herein; (iii) viral particle as disclosed herein; and/or (iv) cell as disclosed herein; (v) esgRNA as disclosed herein; (vi) and a CRISPR/Cas RNA editing fusion protein as disclosed herein; 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 (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.

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 any one of the preceding claims, wherein the ADAR is selected from the group consisting of ADAR1, ADAR2, and ADAR3.

5. The recombinant expression system of claim 4, wherein the catalytically active deaminase domain of ADAR is the catalytically active deaminase domain of ADAR2.

6. The recombinant expression system of claim 5, 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.

7. The recombinant expression system of claim 6, wherein the mutant human catalytically active deaminase domain of ADAR2 comprises a E488Q mutation.

8. The recombinant expression system of any one of the preceding claims, wherein the dCas is nuclease-dead Cas9 (dCas9).

9. The recombinant expression system of claim 8, wherein the dCas9 N-terminal domain is fused to the C-terminus of the catalytically active deaminase domain of ADAR.

10. The recombinant expression system of any one of the preceding claims, wherein the dCas is fused to the catalytically active deaminase domain of ADAR via a linker.

11. The recombinant expression system of claim 10, wherein the linker is a semi-flexible XTEN peptide linker.

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

13. 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.

14. 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.

15. The recombinant expression system of claim 14, 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.

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

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

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

19. The recombinant expression system of claim 18, wherein the RNA polymerase III promoter sequence is a U6 promoter sequence.

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

21. 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.

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

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

24. The recombinant expression system of claim 22, wherein the viral vector is an adeno-associated viral vector (AAV), lentiviral vector, or an adenoviral vector.

25. 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.

26. The vector of claim 25, wherein the vector is a viral vector.

27. The vector of claim 26, wherein the viral vector is an adeno-associated viral vector (AAV), lentiviral vector, or an adenoviral vector.

28. The vector of any one of claims 25 to 27, further comprising an expression control element.

29. A viral particle comprising the vector of any one of claims 25-28.

30. A cell comprising any one of the recombinant expression systems of claims 1 to 24, the vectors of claims 25-28, or the viral particle of claim 29.

31. 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.

32. A CRISPR/Cas RNA editing fusion protein comprising a nuclease-dead CRISPR associated endonuclease (dCas) fused to a catalytically active deaminase domain of ADAR.

33. A method of selective RNA editing comprising administering any one of the recombinant expression systems of claims 1 to 24, the vectors of claims 25-28, or the viral particle of claim 29 to a cell.

34. The method of claim 33, further comprising administering an antisense synthetic oligonucleotide compound comprising alternating 2'OMe RNA and DNA bases (PAMmer).

35. The method of claim 33 or 34, wherein the method is *in vitro* or *in vivo*.

36. 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 24, the vectors of claims 25-28, the viral particle of claim 29, or the cell of claim 30 to a sample and determining its effects.

37. The method of claim 36, wherein the sample is derived from a subject.

38. Use of any one of the recombinant expression systems of claims 1 to 24, the vectors of claims 25-28, the viral particle of claim 29, or the cell of claim 30, in the manufacture of a medicament for treating a disease or condition in a subject, wherein the medicament is to be administered to a subject or a sample isolated from a subject.

39. The use of claim 38, further comprising correction of a G to A mutation in a target RNA.

40. The use of claim 38 or 39, wherein the disease is selected from the group of Hurler's syndrome, Cystic fibrosis, 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.

41. A kit comprising:

(A) one or more of:

(i) recombinant expression system according to any one of claims 1 to 24;

(ii) vector according to any one of claims 25-28;

(iii) viral particle according to claim 29; and/or

(iv) cell according to claim 30;

(v) esgRNA according to claim 31;

(vi) and a CRISPR/Cas RNA editing fusion protein according to claim 32; and

(B) instructions for use.

42. The kit of claim 41, wherein the instructions are for use according to any one of the methods of claims 33-40.