CLAIMS

What is claimed is:

1. A composition comprising a nucleic acid sequence encoding a target RNA knockdown and replacement therapeutic comprising (a) an RNA-binding polypeptide or portion thereof; and (b) a therapeutic protein, wherein the RNA-binding polypeptide binds and cleaves a target RNA or a protein encoded by the target RNA, wherein a pathogenic RNA encoding a pathogenic protein with one or more gain-or-loss-of-function mutations comprises the target RNA, and wherein the therapeutic protein is a replacement protein for the pathogenic protein.
2. A composition comprising a nucleic acid sequence encoding a target RNA knockdown and replacement therapeutic comprising (a) an RNA-binding polypeptide or portion thereof; and (b) a therapeutic protein, wherein the RNA-binding polypeptide binds and cleaves a target rhodopsin RNA or a protein encoded by the target rhodopsin RNA, wherein a pathogenic rhodopsin RNA encoding a pathogenic rhodopsin protein with one or more gain-or-loss-of-function rhodopsin mutations comprises the target rhodopsin RNA, and wherein the therapeutic protein is a wild-type rhodopsin protein.
3. The composition of claim 1, wherein the therapeutic protein is selected from the group consisting of rhodopsin (Retinitis Pigmentosa), PRPF3 (Retinitis Pigmentosa), PRPF31 (autosomal dominant Retinitis Pigmentosa), GRN (FTD), SOD1 (ALS), PMP22 (Charcot Marie Tooth Disease), PABPN1 (Oculopharangeal Muscular Dystrophy), KCNQ4 (Hearing Loss), CLRN1 (Usher Syndrome), APOE2 (Alzheimer's Disease), APOE4 (Alzheimer's Disease), BEST1 (Eye Disease), MYBPC3 (Familial Cardiomyopathy), TNNT2 (Familial Cardiomyopathy), and TNNI3 (Familial Cardiomyopathy).
4. The composition of claim 2 or 3, wherein the therapeutic protein is rhodopsin.
5. The composition of claim 3 or 4, wherein rhodopsin is human rhodopsin.
6. The composition of claim 4, wherein the therapeutic rhodopsin is "hardened" rhodopsin.
7. The composition of claim 1 or 2, wherein the RNA binding protein comprises a Pumilio and FBF (PUF) protein.

8. The composition of claim 1 or 2, wherein the RNA binding protein comprises a Pumilio-based assembly (PUMBY) protein.
9. The composition of claim 2, wherein the rhodopsin target RNA sequence comprises CAACGAGTCTTTTGTCATCTACATGT (SEQ ID NO: 462), CGCCAGCGTGGCATTCTACATCTTCA (SEQ ID NO: 463), or CATCTATATCATGATGAACAAGCAGT (SEQ ID NO: 464).
10. The composition of claim 9, wherein the rhodopsin target RNA encodes an amino acid comprising YASVAFYIFT (SEQ ID NO: 486) at position 268 to 277.
11. The composition of claim 5, wherein the "hardened" rhodopsin is encoded by a nucleic acid sequence which does not comprise the rhodopsin target RNA comprising GCCAGCGTGGCATTCTACATCTTC (SEQ ID NO: 406).
12. The composition of claim 11, wherein the "hardened" rhodopsin is encoded by a nucleic acid sequence comprising GCTTCCGTAGCTTTTTATATTTTT (SEQ ID NO: 408).
13. The composition of claim 1 or 2, wherein the nucleic acid sequence comprises at least one promoter.
14. The composition of claim 8, wherein the at least one promoter is a constitutive promoter or a tissue-specific promoter.
15. The composition of claim 9, wherein the at least one promoter is selected from the group consisting of opsin promoter, EFS promoter, and both.
16. The composition of claim 1 or 2, wherein the nucleic acid sequence comprises two promoters.
17. A vector comprising the composition of any one of claims 1 or 2.
18. The vector of claim 17, wherein the vector is selected from the group consisting of: adeno-associated virus, retrovirus, lentivirus, adenovirus, nanoparticle, micelle, liposome, lipoplex, polymersome, polyplex, and dendrimer.
19. A cell comprising the vector of claim 17.
20. The composition of any one of claims 1 or 2, wherein the RNA-binding polypeptide is a first RNA-binding polypeptide, and wherein the nucleic acid sequence encodes a second RNA-binding polypeptide which binds RNA in a manner in which it associates with RNA.
21. The composition of claim 20, wherein the second RNA-binding polypeptide associates with RNA in a manner in which it cleaves RNA.

22. The composition of claim 20, wherein the second RNA-binding polypeptide is selected from the group consisting of: RNAse1, RNAse4, RNAse6, RNAse7, RNAse8, RNAse2, RNAse6PL, RNAseL, RNAseT2, RNAse11, RNAseT2-like, NOB1, ENDOV, ENDOG, ENDOD1, hFEN1, hSLFN14, hLACTB2, APEX2, ANG, HRSP12, ZC3H12A, RIDA, PDL6, NTHL, KIAA0391, APEX1, AGO2, EXOG, ZC3H12D, ERN2, PELO, YBEY, CPSF4L, hCG_2002731, ERCC1, RAC1, RAA1, RAB1, DNA2, FLJ35220, FLJ13173, ERCC4, Rnase1(K41R), Rnase1(K41R, D121E), Rnase1(K41R, D121E, H119N), Rnase1(H119N), Rnase1(R39D, N67D, N88A, G89D, R91D, H119N), Rnase1(R39D, N67D, N88A, G89D, R91D, H119N, K41R, D121E), Rnase1(R39D, N67D, N88A, G89D, R91D), TENM1, TENM2, RNAseK, TALEN, ZNF638, and hSMG6.
23. The composition of claim 22, wherein the second RNA-binding polypeptide is ZC3H12A.
24. A method for reducing the level of expression of a pathogenic target RNA molecule or a protein encoded by the pathogenic RNA molecule and replacing gain-or-loss-of-function mutations caused by the pathogenic target RNA with a therapeutic replacement protein, the method comprising contacting the composition of claim 1 or 2 and the pathogenic target RNA molecule comprising a target RNA sequence under conditions suitable for binding of the RNA binding protein to the target RNA sequence, wherein the level of expression of the pathogenic target RNA is reduced, and wherein the expression of the pathogenic target RNA is replaced with expression of a therapeutic replacement protein.
25. An adeno-associated viral (AAV) vector comprising the composition of claim 1.