

SUMMARY

In one aspect, the present disclosure provides a vector stuffer sequence comprising a nucleic acid of about 1300 to about 2300 nucleotides in length and having at least 75% identity to any one of: SEQ ID NOS:48, 45-47, and 49-51; nucleotides 489-2185 of any one of SEQ ID NOS:13-16; nucleotides 342-2222 of SEQ ID NO:17; nucleotides 488-2177 of SEQ ID NO:18; nucleotides 489-2177 of SEQ ID NO:19; nucleotides 711-2187 of SEQ ID NO:20; nucleotides 711-2187 of SEQ ID NO:21; nucleotides 488-2177 of SEQ ID NO:22; nucleotides 711-2187 of SEQ ID NO:23; nucleotides 711-2187 of SEQ ID NO:24; nucleotides 252-2132 of SEQ ID NO:25; nucleotides 252-2132 of SEQ ID NO:26; nucleotides 252-2132 of SEQ ID NO:27; nucleotides 252-2132 of SEQ ID NO:28; nucleotides 357-2237 of SEQ ID NO:29; nucleotides 358-2195 of SEQ ID NO:30; nucleotides 580-2187 of SEQ ID NO:31; nucleotides 580-2196 of SEQ ID NO:32; nucleotides 357-2237 of SEQ ID NO:33; nucleotides 358-2195 of SEQ ID NO:34; nucleotides 580-2187 of SEQ ID NO:35; nucleotides 580-2196 of SEQ ID NO:36; nucleotides 348-2228 of SEQ ID NO:37; nucleotides 349-2229 of SEQ ID NO:38; nucleotides 349-2186 of SEQ ID NO:39; nucleotides 571-2178 of SEQ ID NO:40; nucleotides 571-2178 of SEQ ID NO:41; nucleotides 348-2228 of SEQ ID NO:42; nucleotides 349-2186 of SEQ ID NO:43; nucleotides 571-2178 of SEQ ID NO:44; nucleotides 349-2229 of SEQ ID NO:78; nucleotides 358-2238 of SEQ ID NO:79; nucleotides 342-2222 of SEQ ID NO:80; and nucleotides 342-2222 of SEQ ID NO:81.

In another aspect, the present disclosure provides a recombinant AAV vector comprising the vector stuffer sequence as described herein. In yet another aspect, the present disclosure provides a rAAV particle comprising the rAAV vector as described herein. In yet another aspect, the present disclosure provides a use of the rAAV particle as described herein in the manufacture of a medicament for treating a subject having or at risk of developing a neurodegenerative disease.

CLAIMS

1. A vector stuffer sequence comprising a nucleic acid of about 1300 to about 2300 nucleotides in length and having at least 75% identity to any one of: SEQ ID NOS:48, 45-47, and 49-51; nucleotides 489-2185 of any one of SEQ ID NOS:13-16;1; nucleotides 342-2222 of SEQ ID NO:17; nucleotides 488-2177 of SEQ ID NO:18; nucleotides 489-2177 of SEQ ID NO:19; nucleotides 711-2187 of SEQ ID NO:20; nucleotides 711-2187 of SEQ ID NO:21; nucleotides 488-2177 of SEQ ID NO:22; nucleotides 711-2187 of SEQ ID NO:23; nucleotides 711-2187 of SEQ ID NO:24; nucleotides 252-2132 of SEQ ID NO:25; nucleotides 252-2132 of SEQ ID NO:26; nucleotides 252-2132 of SEQ ID NO:27; nucleotides 252-2132 of SEQ ID NO:28; nucleotides 357-2237 of SEQ ID NO:29; nucleotides 358-2195 of SEQ ID NO:30; nucleotides 580-2187 of SEQ ID NO:31; nucleotides 580-2196 of SEQ ID NO:32; nucleotides 357-2237 of SEQ ID NO:33; nucleotides 358-2195 of SEQ ID NO:34; nucleotides 580-2187 of SEQ ID NO:35; nucleotides 580-2196 of SEQ ID NO:36; nucleotides 348-2228 of SEQ ID NO:37; nucleotides 349-2229 of SEQ ID NO:38; nucleotides 349-2186 of SEQ ID NO:39; nucleotides 571-2178 of SEQ ID NO:40; nucleotides 571-2178 of SEQ ID NO:41; nucleotides 348-2228 of SEQ ID NO:42; nucleotides 349-2186 of SEQ ID NO:43; nucleotides 571-2178 of SEQ ID NO:44; nucleotides 349-2229 of SEQ ID NO:78; nucleotides 358-2238 of SEQ ID NO:79; nucleotides 342-2222 of SEQ ID NO:80; and nucleotides 342-2222 of SEQ ID NO:81.

2. The vector stuffer sequence of claim 1, wherein the nucleic acid is about 1500-2000 nucleotides in length.

3. The vector stuffer sequence of claim 1 or 2, wherein the nucleic acid is about 1600 to 1900 nucleotides in length.

4. The vector stuffer sequence of any one of claims 1-3, wherein the nucleic acid has at least 80% identity to any one of: SEQ ID NOS:48, 45-47, and 49-51;

nucleotides 489-2185 of any one of SEQ ID NOS:13-16; nucleotides 342-2222 of SEQ ID NO:17; nucleotides 488-2177 of SEQ ID NO:18; nucleotides 489-2177 of SEQ ID NO:19; nucleotides 711-2187 of SEQ ID NO:20; nucleotides 711-2187 of SEQ ID NO:21; nucleotides 488-2177 of SEQ ID NO:22; nucleotides 711-2187 of SEQ ID NO:23; nucleotides 711-2187 of SEQ ID NO:24; nucleotides 252-2132 of SEQ ID NO:25; nucleotides 252-2132 of SEQ ID NO:26; nucleotides 252-2132 of SEQ ID NO:27; nucleotides 252-2132 of SEQ ID NO:28; nucleotides 357-2237 of SEQ ID NO:29; nucleotides 358-2195 of SEQ ID NO:30; nucleotides 580-2187 of SEQ ID NO:31; nucleotides 580-2196 of SEQ ID NO:32; nucleotides 357-2237 of SEQ ID NO:33; nucleotides 358-2195 of SEQ ID NO:34; nucleotides 580-2187 of SEQ ID NO:35; nucleotides 580-2196 of SEQ ID NO:36; nucleotides 348-2228 of SEQ ID NO:37; nucleotides 349-2229 of SEQ ID NO:38; nucleotides 349-2186 of SEQ ID NO:39; nucleotides 571-2178 of SEQ ID NO:40; nucleotides 571-2178 of SEQ ID NO:41; nucleotides 348-2228 of SEQ ID NO:42; nucleotides 349-2186 of SEQ ID NO:43; nucleotides 571-2178 of SEQ ID NO:44; and nucleotides 349-2229 of SEQ ID NO:78; nucleotides 358-2238 of SEQ ID NO:79; nucleotides 342-2222 of SEQ ID NO:80; and nucleotides 342-2222 of SEQ ID NO:81.

5. The vector stuffer sequence of any one of claims 1-4, wherein the nucleic acid has at least 85% identity to any one of: SEQ ID NOS:48, 45-47, and 49-51; nucleotides 489-2185 of any one of SEQ ID NOS:13-16; nucleotides 342-2222 of SEQ ID NO:17; nucleotides 488-2177 of SEQ ID NO:18; nucleotides 489-2177 of SEQ ID NO:19; nucleotides 711-2187 of SEQ ID NO:20; nucleotides 711-2187 of SEQ ID NO:21; nucleotides 488-2177 of SEQ ID NO:22; nucleotides 711-2187 of SEQ ID NO:23; nucleotides 711-2187 of SEQ ID NO:24; nucleotides 252-2132 of SEQ ID NO:25; nucleotides 252-2132 of SEQ ID NO:26; nucleotides 252-2132 of SEQ ID NO:27; nucleotides 252-2132 of SEQ ID NO:28; nucleotides 357-2237 of SEQ ID NO:29; nucleotides 358-2195 of SEQ ID NO:30; nucleotides 580-2187 of SEQ ID NO:31; nucleotides 580-2196 of SEQ ID NO:32; nucleotides 357-2237 of SEQ ID NO:33; nucleotides 358-2195 of SEQ ID NO:34; nucleotides 580-2187 of SEQ ID

NO:35; nucleotides 580-2196 of SEQ ID NO:36; nucleotides 348-2228 of SEQ ID NO:37; nucleotides 349-2229 of SEQ ID NO:38; nucleotides 349-2186 of SEQ ID NO:39; nucleotides 571-2178 of SEQ ID NO:40; nucleotides 571-2178 of SEQ ID NO:41; nucleotides 348-2228 of SEQ ID NO:42; nucleotides 349-2186 of SEQ ID NO:43; nucleotides 571-2178 of SEQ ID NO:44; nucleotides 349-2229 of SEQ ID NO:78; nucleotides 358-2238 of SEQ ID NO:79; nucleotides 342-2222 of SEQ ID NO:80; and nucleotides 342-2222 of SEQ ID NO:81.

6. The vector stuffer sequence of any one of claims 1-5, wherein the nucleic acid has at least 90% identity to any one of: SEQ ID NOS:[48](#), [45-47](#), and [49](#)-51; nucleotides 489-2185 of any one of SEQ ID NOS:13-16; nucleotides 342-2222 of SEQ ID NO:17; nucleotides 488-2177 of SEQ ID NO:18; nucleotides 711-2187 of SEQ ID NO:21; nucleotides 488-2177 of SEQ ID NO:22; nucleotides 711-2187 of SEQ ID NO:23; nucleotides 711-2187 of SEQ ID NO:24; nucleotides 252-2132 of SEQ ID NO:25; nucleotides 252-2132 of SEQ ID NO:26; nucleotides 252-2132 of SEQ ID NO:27; nucleotides 252-2132 of SEQ ID NO:28; nucleotides 357-2237 of SEQ ID NO:29; nucleotides 358-2195 of SEQ ID NO:30; nucleotides 580-2187 of SEQ ID NO:31; nucleotides 580-2196 of SEQ ID NO:32; nucleotides 357-2237 of SEQ ID NO:33; nucleotides 358-2195 of SEQ ID NO:34; nucleotides 580-2187 of SEQ ID NO:35; nucleotides 580-2196 of SEQ ID NO:36; nucleotides 348-2228 of SEQ ID NO:37; nucleotides 349-2229 of SEQ ID NO:38; nucleotides 349-2186 of SEQ ID NO:39; nucleotides 571-2178 of SEQ ID NO:40; nucleotides 571-2178 of SEQ ID NO:41; nucleotides 348-2228 of SEQ ID NO:42; nucleotides 349-2186 of SEQ ID NO:43; and nucleotides 571-2178 of SEQ ID NO:44; nucleotides 349-2229 of SEQ ID NO:78; nucleotides 358-2238 of SEQ ID NO:79; nucleotides 342-2222 of SEQ ID NO:80; and nucleotides 342-2222 of SEQ ID NO:81.

7. The vector stuffer sequence of any one of claims 1-6, wherein the nucleic acid has at least 95% identity to any one of: SEQ ID NOS:[48](#), [45-47](#), and [49](#)-51; nucleotides 489-2185 of any one of SEQ ID NOS:13-16; nucleotides 342-2222 of SEQ

ID NO:17; nucleotides 488-2177 of SEQ ID NO:18; nucleotides 711-2187 of SEQ ID NO:21; nucleotides 488-2177 of SEQ ID NO:22; nucleotides 711-2187 of SEQ ID NO:23; nucleotides 711-2187 of SEQ ID NO:24; nucleotides 252-2132 of SEQ ID NO:25; nucleotides 252-2132 of SEQ ID NO:26; nucleotides 252-2132 of SEQ ID NO:27; nucleotides 252-2132 of SEQ ID NO:28; nucleotides 357-2237 of SEQ ID NO:29; nucleotides 358-2195 of SEQ ID NO:30; nucleotides 580-2187 of SEQ ID NO:31; nucleotides 580-2196 of SEQ ID NO:32; nucleotides 357-2237 of SEQ ID NO:33; nucleotides 358-2195 of SEQ ID NO:34; nucleotides 580-2187 of SEQ ID NO:35; nucleotides 580-2196 of SEQ ID NO:36; nucleotides 348-2228 of SEQ ID NO:37; nucleotides 349-2229 of SEQ ID NO:38; nucleotides 349-2186 of SEQ ID NO:39; nucleotides 571-2178 of SEQ ID NO:40; nucleotides 571-2178 of SEQ ID NO:41; nucleotides 348-2228 of SEQ ID NO:42; nucleotides 349-2186 of SEQ ID NO:43; nucleotides 571-2178 of SEQ ID NO:44; nucleotides 349-2229 of SEQ ID NO:78; nucleotides 358-2238 of SEQ ID NO:79; and nucleotides 342-2222 of SEQ ID NO:80; nucleotides 342-2222 of SEQ ID NO:81.

8. The vector stuffer sequence of any one of claims 1-7, wherein the nucleic acid has at least 97% identity to any one of: SEQ ID NOS: 48, 45-47, and 49-51; nucleotides 489-2185 of any one of SEQ ID NOS:13-16; nucleotides 342-2222 of SEQ ID NO:17; nucleotides 488-2177 of SEQ ID NO:18; nucleotides 711-2187 of SEQ ID NO:21; nucleotides 488-2177 of SEQ ID NO:22; nucleotides 711-2187 of SEQ ID NO:23; nucleotides 711-2187 of SEQ ID NO:24; nucleotides 252-2132 of SEQ ID NO:25; nucleotides 252-2132 of SEQ ID NO:26; nucleotides 252-2132 of SEQ ID NO:27; nucleotides 252-2132 of SEQ ID NO:28; nucleotides 357-2237 of SEQ ID NO:29; nucleotides 358-2195 of SEQ ID NO:30; nucleotides 580-2187 of SEQ ID NO:31; nucleotides 580-2196 of SEQ ID NO:32; nucleotides 357-2237 of SEQ ID NO:33; nucleotides 358-2195 of SEQ ID NO:34; nucleotides 580-2187 of SEQ ID NO:35; nucleotides 580-2196 of SEQ ID NO:36; nucleotides 348-2228 of SEQ ID NO:37; nucleotides 349-2229 of SEQ ID NO:38; nucleotides 349-2186 of SEQ ID NO:39; nucleotides 571-2178 of SEQ ID NO:40; nucleotides 571-2178 of SEQ ID

NO:41; nucleotides 348-2228 of SEQ ID NO:42; nucleotides 349-2186 of SEQ ID NO:43; and nucleotides 571-2178 of SEQ ID NO:44; nucleotides 349-2229 of SEQ ID NO:78; nucleotides 358-2238 of SEQ ID NO:79; nucleotides 342-2222 of SEQ ID NO:80; and nucleotides 342-2222 of SEQ ID NO:81.

9. The vector stuffer sequence of any one of claims 1-8, wherein the nucleic acid comprises or consists of any one of: SEQ ID NOS:[48](#), [45-47](#), and [48](#)-51; nucleotides 489-2185 of any one of SEQ ID NOS:13-16; nucleotides 342-2222 of SEQ ID NO:17; nucleotides 488-2177 of SEQ ID NO:18; nucleotides 711-2187 of SEQ ID NO:21; nucleotides 488-2177 of SEQ ID NO:22; nucleotides 711-2187 of SEQ ID NO:23; nucleotides 711-2187 of SEQ ID NO:24; nucleotides 252-2132 of SEQ ID NO:25; nucleotides 252-2132 of SEQ ID NO:26; nucleotides 252-2132 of SEQ ID NO:27; nucleotides 252-2132 of SEQ ID NO:28; nucleotides 357-2237 of SEQ ID NO:29; nucleotides 358-2195 of SEQ ID NO:30; nucleotides 580-2187 of SEQ ID NO:31; nucleotides 580-2196 of SEQ ID NO:32; nucleotides 357-2237 of SEQ ID NO:33; nucleotides 358-2195 of SEQ ID NO:34; nucleotides 580-2187 of SEQ ID NO:35; nucleotides 580-2196 of SEQ ID NO:36; nucleotides 348-2228 of SEQ ID NO:37; nucleotides 349-2229 of SEQ ID NO:38; nucleotides 349-2186 of SEQ ID NO:39; nucleotides 571-2178 of SEQ ID NO:40; nucleotides 571-2178 of SEQ ID NO:41; nucleotides 348-2228 of SEQ ID NO:42; nucleotides 349-2186 of SEQ ID NO:43; and nucleotides 571-2178 of SEQ ID NO:44; nucleotides 349-2229 of SEQ ID NO:78; nucleotides 358-2238 of SEQ ID NO:79; nucleotides 342-2222 of SEQ ID NO:80; and nucleotides 342-2222 of SEQ ID NO:81.

10. The vector stuffer sequence of any one of claims 1-9, wherein the vector is an adeno-associated virus (AAV) vector, optionally wherein the AAV vector is self-complementary.

11. The vector stuffer sequence of any one of claims 1-10, wherein the vector stuffer sequence is adjacent to an expression construct comprising a heterologous nucleic acid sequence.

12. The vector stuffer sequence of claim 11, wherein the heterologous nucleic acid sequence encodes a therapeutic agent.

13. The vector stuffer sequence of claim 12, wherein the therapeutic agent comprises a nucleic acid encoding an inhibitory nucleic acid.

14. The vector stuffer sequence of claim 13, wherein the inhibitory nucleic acid comprises a miRNA, siRNA, ~~miRNA~~, shRNA, or dsRNA.

15. The vector stuffer sequence of claim 14, wherein the inhibitory nucleic acid comprises an miRNA targeting a neurodegenerative disease related gene, optionally wherein the neurodegenerative disease is amyotrophic lateral sclerosis, spinocerebellar ataxia-2, ~~amyotrophic lateral sclerosis~~, frontotemporal dementia, primary lateral sclerosis, progressive muscular atrophy, limbic-predominant age-related TDP-43 encephalopathy, chronic traumatic encephalopathy, dementia with Lewy bodies, corticobasal degeneration, progressive supranuclear palsy (PSP), dementia Parkinsonism ALS complex of guam (G-PDC), Pick's disease, hippocampal sclerosis, Huntington's disease, Parkinson's disease, or Alzheimer's disease.

16. The vector stuffer sequence of claim 15, wherein the neurodegenerative disease is a polyglutamine repeat disease.

17. The vector stuffer sequence of any one of claim 13-16, wherein the inhibitory nucleic acid targets ATXN2.

18. The vector stuffer sequence of any one of claims 13-17, wherein the heterologous nucleic acid encodes an artificial miRNA comprising:

- (a) a guide sequence selected from SEQ ID NOS:1-4 and 71;
- (b) a guide sequence provided in SEQ ID NO:1 and a passenger sequence provided by SEQ ID NO:5; a guide sequence provided in SEQ ID NO:2 and a passenger sequence provided by SEQ ID NO:6; a guide sequence provided in SEQ ID NO:3 and a passenger sequence provided by SEQ ID NO:7; a guide sequence provided by SEQ ID NO:4 and a passenger sequence provided by SEQ ID NO:8; or a guide sequence provided by SEQ ID NO:71 and a passenger sequence provided by SEQ ID NO:72; or
- (c) a sequence provided by any one of SEQ ID NOS:9-12 and 73.

19. The vector stuffer sequence of any one of claims 11-18, wherein the expression construct comprises a promoter, a polyadenylation signal, a termination signal, or any combination thereof.

20. The vector stuffer sequence of claim 19, wherein the promoter is a H1 promoter, optionally wherein:

- (a) the H1 promoter is a H1 long promoter comprising SEQ ID NO:52; nucleotides 113-343 of any one of SEQ ID NOS:13-16 and 18-24;
- (b) the H1 promoter is a H1 promoter comprising SEQ ID NO:53; or
- (c) the H1 promoter is a H1 short promoter comprising SEQ ID NO:54 or nucleotides 113-203 of any one of SEQ ID NOS:17, 25-28, and 37-44.

21. The vector stuffer sequence of claim 19 or 20, wherein the termination signal is a SV40 termination signal, optionally wherein the SV40 termination signal comprises the sequence provided by SEQ ID NO:77.

22. A recombinant AAV vector comprising the vector stuffer sequence of any one of claims 1-21.

23. The recombinant AAV vector of claim 22, wherein the AAV vector is self-complementary.

24. The recombinant AAV vector of claim 22 or 23, wherein the AAV vector comprises a 5' inverted terminal repeat (ITR) and a 3' ITR flanking the expression construct and vector stuffer sequence.

25. The recombinant AAV vector of claim 24, wherein the 5' ITR and 3' ITR are obtained from an AAV serotype selected from the group consisting of: AAV1, AAV2, AAV5, AAV6, AAV6.2, AAV7, AAV8, AAV9, AAVRh10, AAV11, and variants thereof.

26. The recombinant AAV vector of claim 24 or 25, wherein one of the 5' ITR and 3' ITR lacks a functional terminal resolution site.

27. The recombinant AAV vector of claim 26, wherein the 5' ITR lacks a functional terminal resolution site.

28. The recombinant AAV vector of any one of claims 24-27, wherein:

(a) the 5' ITR comprises SEQ ID NO:57 or nucleotides 1-106 of any one of SEQ ID NOS:13-24, 29-44, and 78-82; and/or

(b) the 3' ITR comprises SEQ ID NO:58; nucleotides 2192-2358 of any one of SEQ ID NOS:13-16; nucleotides 2229-2395 of SEQ ID NO:17; nucleotides 2184-2350 of SEQ ID NO:18; nucleotides 2214-2358 of any one of SEQ ID NOS:13-16, nucleotides 2251-2395 of SEQ ID NO:17, nucleotides 2206-2350 of SEQ ID NO:18, nucleotides 2206-2350 of SEQ ID NO:19; nucleotides 2216-2360 of SEQ ID NO:20; nucleotides 2216-2360 of SEQ ID NO:21; nucleotides 2206-2350 of SEQ ID NO:22; nucleotides 2216-2360 of SEQ ID NO:23; nucleotides 2216-2360 of SEQ ID NO:24; nucleotides 2161-2305 of SEQ ID NO:25; nucleotides 2161-2305 of SEQ ID NO:26; nucleotides 2161-2305 of SEQ ID NO:27; nucleotides 2161-2305 of SEQ ID

NO:28; nucleotides 2266-2410 of SEQ ID NO:29; nucleotides 2224-2368 of SEQ ID NO:30; nucleotides 2216-2360 of SEQ ID NO:31; nucleotides 2225-2369 of SEQ ID NO:32; nucleotides 2266-2410 of SEQ ID NO:33; nucleotides 2224-2368 of SEQ ID NO:34; nucleotides 2216-2360 of SEQ ID NO:35; nucleotides 2225-2369 of SEQ ID NO:36; nucleotides 2257-2401 of SEQ ID NO:37; nucleotides 2258-2402 of SEQ ID NO:38; nucleotides 2215-2359 of SEQ ID NO:39; nucleotides 2207-2351 of SEQ ID NO:40; nucleotides 2207-2351 of SEQ ID NO:41; nucleotides 2257-2401 of SEQ ID NO:42; nucleotides 2215-2359 of SEQ ID NO:43; nucleotides 2207-2351 of SEQ ID NO:44; nucleotides 2258-2402 of SEQ ID NO:78; nucleotides 2267-2411 of SEQ ID NO:79; nucleotides 2251-2395 of SEQ ID NO:80; nucleotides 2251-2395 of SEQ ID NO:81; or nucleotides 2187-2331 of SEQ ID NO:82.

29. The recombinant AAV vector of any one of claims 22-28, comprising:

- (a) the nucleotide sequence of any one of SEQ ID NOS:13-24, 29-44, and 78-80;
- (b) the nucleotide sequence of SEQ ID NO:13 wherein nucleotides 344-481 are substituted with a sequence encoding a miRNA of interest;
- (b) the nucleotide sequence of SEQ ID NO:14 wherein nucleotides 344-481 are substituted with a sequence encoding a miRNA of interest;
- (c) the nucleotide sequence of SEQ ID NO:15 wherein nucleotides 344-481 are substituted with a sequence encoding a miRNA of interest;
- (d) the nucleotide sequence of SEQ ID NO:16 wherein nucleotides 344-481 are substituted with a sequence encoding a miRNA of interest;
- (e) the nucleotide sequence of SEQ ID NO:17 wherein nucleotides 204-335 are substituted with a sequence encoding a miRNA of interest;
- (f) the nucleotide sequence of SEQ ID NO:18 wherein nucleotides 344-481 are substituted with a sequence encoding a miRNA of interest;
- (g) the nucleotide sequence of SEQ ID NO:19, wherein nucleotides 344-481 are substituted with a sequence encoding a miRNA of interest;

- (h) the nucleotide sequence of SEQ ID NO:20, wherein nucleotides 344-481 are substituted with a sequence encoding a miRNA of interest;
- (i) the nucleotide sequence of SEQ ID NO:21, wherein nucleotides 344-481 are substituted with a sequence encoding a miRNA of interest;
- (j) the nucleotide sequence of SEQ ID NO:22, wherein nucleotides 344-481 are substituted with a sequence encoding a miRNA of interest;
- (k) the nucleotide sequence of SEQ ID NO:23, wherein nucleotides 344-481 are substituted with a sequence encoding a miRNA of interest;
- (l) the nucleotide sequence of SEQ ID NO:24, wherein nucleotides 344-481 are substituted with a sequence encoding a miRNA of interest;
- (m) the nucleotide sequence of SEQ ID NO:29, wherein nucleotides 213-350 are substituted with a sequence encoding a miRNA of interest;
- (n) the nucleotide sequence of SEQ ID NO:30, wherein nucleotides 213-350 are substituted with a sequence encoding a miRNA of interest;
- (o) the nucleotide sequence of SEQ ID NO:31, wherein nucleotides 213-350 are substituted with a sequence encoding a miRNA of interest;
- (p) the nucleotide sequence of SEQ ID NO:32, wherein nucleotides 213-350 are substituted with a sequence encoding a miRNA of interest;
- (q) the nucleotide sequence of SEQ ID NO:33, wherein nucleotides 213-350 are substituted with a sequence encoding a miRNA of interest;
- (r) the nucleotide sequence of SEQ ID NO:34, wherein nucleotides 213-350 are substituted with a sequence encoding a miRNA of interest;
- (s) the nucleotide sequence of SEQ ID NO:35, wherein nucleotides 213-350 are substituted with a sequence encoding a miRNA of interest;
- (t) the nucleotide sequence of SEQ ID NO:36, wherein nucleotides 213-350 are substituted with a sequence encoding a miRNA of interest;
- (u) the nucleotide sequence of SEQ ID NO:37, wherein nucleotides 204-341 are substituted with a sequence encoding a miRNA of interest;
- (v) the nucleotide sequence of SEQ ID NO:38, wherein nucleotides 204-341 are substituted with a sequence encoding a miRNA of interest;

(w) the nucleotide sequence of SEQ ID NO:39, wherein nucleotides 204-341 are substituted with a sequence encoding a miRNA of interest;

(x) the nucleotide sequence of SEQ ID NO:40, wherein nucleotides 204-341 are substituted with a sequence encoding a miRNA of interest;

(y) the nucleotide sequence of SEQ ID NO:41, wherein nucleotides 204-341 are substituted with a sequence encoding a miRNA of interest;

(z) the nucleotide sequence of SEQ ID NO:42, wherein nucleotides 204-341 are substituted with a sequence encoding a miRNA of interest;

(aa) the nucleotide sequence of SEQ ID NO:43, wherein nucleotides 204-341 are substituted with a sequence encoding a miRNA of interest;

(bb) the nucleotide sequence of SEQ ID NO:44, wherein nucleotides 204-341 are substituted with a sequence encoding a miRNA of interest;

(cc) the nucleotide sequence of SEQ ID NO:78, wherein nucleotides 204-342 are substituted with a sequence encoding an artificial miRNA of interest;

(dd) the nucleotide sequence of SEQ ID NO:79, wherein nucleotides 213-351 are substituted with a sequence encoding an artificial miRNA of interest;

(ee) the nucleotide sequence set forth in SEQ ID NO:80, wherein nucleotides 204-335 are substituted with a sequence encoding an artificial miRNA of interest; or

(ff) the nucleotide sequence set forth in SEQ ID NO:81, wherein nucleotides 204-335 are substituted with a sequence encoding an artificial miRNA of interest.

30. A rAAV particle comprising the rAAV vector of any one of claims 22-29.

31. The rAAV particle of claim 30, wherein the rAAV particle comprises a capsid protein.

32. The rAAV particle of claim 31, wherein the capsid protein is capable of crossing the blood-brain barrier.

33. The rAAV particle of claim 31 or 32, wherein the capsid protein is an AAV9 capsid protein.

34. ~~A method of delivering a therapeutic agent to a subject, comprising administering to the subject~~Use of the rAAV particle of any one of claims ~~28-32~~30-33 in the manufacture of a medicament for treating a subject having or at risk of developing a neurodegenerative disease.

~~35. The method of claim 34, wherein subject has a neurodegenerative disease or is at risk of developing a neurodegenerative disease.~~

~~36~~35. The ~~method-use~~ of claim ~~34-or 35~~, wherein the medicament is to be directly administration comprises direct injection to~~injected into~~ the CNS of the subject.

~~37~~36. The ~~method-use~~ of claim ~~36~~35, wherein the direct injection is intracerebral injection, intraparenchymal injection, intrathecal injection, intrastriatal injection, subpial injection, or any combination thereof.

~~38~~37. The ~~method-use~~ of claim ~~36~~35, wherein the direct injection is direct injection to the cerebrospinal fluid (CSF) of the subject, optionally wherein the direct injection is intracisternal injection, intraventricular injection, and/or intralumbar injection.

~~39~~38. The ~~method-use~~ of any one of claims ~~35-38~~34-37, wherein the neurodegenerative disease is amyotrophic lateral sclerosis, spinocerebellar ataxia-2, ~~amyotrophic lateral sclerosis~~, frontotemporal dementia, primary lateral sclerosis, progressive muscular atrophy, limbic-predominant age-related TDP-43 encephalopathy, chronic traumatic encephalopathy, dementia with Lewy bodies, corticobasal degeneration, progressive supranuclear palsy (PSP), dementia Parkinsonism ALS

complex of guam (G-PDC), Pick's disease, hippocampal sclerosis, Huntington's disease, Parkinson's disease, or Alzheimer's disease.