

**AAV-BASED ANTI-GA THERAPY****RELATED APPLICATIONS**

The application claims the benefit under 35 U.S.C. 119(e) of U.S. Provisional  
5 Application number 63/608,667 filed on December 11, 2023, U.S. Provisional Application  
number 63/554,852 filed on February 16, 2024, and U.S. Provisional Application number  
63/645,222 filed on May 10, 2024, each of which is herein incorporated by reference in its  
entirety.

10 **REFERENCE TO AN ELECTRONIC SEQUENCE LISTING**

The contents of the electronic sequence listing (U120270132WO00-SEQ-KZM.xml;  
Size: 91,478 bytes; and Date of Creation: December 5, 2024) is herein incorporated by reference  
in its entirety.

15 **GOVERNMENT SUPPORT**

This invention was made with government support under grant number HT9425-23-1-  
0287, awarded by the U.S. Army Medical Research Acquisition Activity. The government has  
certain rights in the invention.

20 **BACKGROUND**

Microsatellite repeat expansions are known to cause more than sixty neurodegenerative  
disorders. Molecular features common to many of these disorders include the accumulation of  
RNA foci containing sense and antisense expansion transcripts and the accumulation of proteins  
from repeat-associated non-AUG (RAN) translation. RAN translation can occur across a broad  
25 range of repeat lengths from pre-mutation lengths (~20 – 40 repeats) to full expansions (greater  
than 11,000 repeats). There is growing evidence that RAN proteins are toxic and contribute to a  
growing number of diseases and disorders, including, but not limited to, amyotrophic lateral  
sclerosis (ALS), frontotemporal dementia (FTD), myotonic dystrophy type 1 (DM1), myotonic  
dystrophy type 2 (DM2), Huntington's disease (HD), Alzheimer's disease (AD), and Fragile X  
30 Tremor Ataxia Syndrome (FXTAS).

**SUMMARY**

Aspects of the disclosure relate to compositions and methods for the diagnosis and/or  
treatment of certain neurodegenerative diseases, for example those diseases associated with  
35 repeat-associated non-ATG (RAN) translation proteins, such as amyotrophic lateral sclerosis

(ALS). The disclosure is based, in part, on viral vectors, such as recombinant adeno-associated virus (rAAV) vectors and rAAV particles encoding antibodies (e.g., monoclonal antibodies) and antigen-binding fragments thereof (e.g., single-chain variable fragments, scFvs) that bind to poly-Glycine-Alanine (poly(GA)) RAN proteins. In some embodiments, the disclosure relates to methods of reducing RAN protein (e.g., poly(GA) RAN protein) aggregation in a subject by administering the rAAV vectors or rAAV particles to the subject. In some embodiments, the disclosure relates to methods of treating a RAN protein-associated disease by administering to a subject in need thereof the rAAVs.

Accordingly, In some aspects, the disclosure provides a recombinant adeno-associated virus (rAAV) vector comprising a nucleic acid sequence encoding an anti-poly(GA) RAN protein antibody or antigen-binding fragment thereof, flanked by adeno-associated virus (AAV) inverted terminal repeats (ITRs).

In some embodiments, the AAV ITRs are AAV2 ITRs.

In some embodiments, the anti-poly(GA) RAN protein antibody or antigen-binding fragment thereof comprises a heavy chain variable region (VH) comprising: a CDR1 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 1; a CDR2 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 2; and/or a CDR3 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 3.

In some embodiments, the anti-poly(GA) RAN protein antibody or antigen-binding fragment comprises a light chain variable region (VL) comprising: a CDR1 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 4; a CDR2 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 5; and/or a CDR3 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 6.

In some embodiments, the anti-poly(GA) RAN protein antibody or antigen-binding fragment comprises a variable heavy chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 7.

In some embodiments, the anti-poly(GA) RAN protein antibody or antigen-binding fragment comprises a variable light chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 12.

In some embodiments, the anti-poly(GA) RAN protein antibody or antigen-binding fragment comprises a heavy chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 8 or 10.

In some embodiments, the anti-poly(GA) RAN protein antibody or antigen-binding fragment comprises a light chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 13 or 15.

In some embodiments, the anti-poly(GA) RAN protein antibody or antigen-binding fragment comprises a heavy chain variable region (VH) comprising: a CDR1 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 20; a CDR2 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21; and/or a CDR3 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22.

In some embodiments, the anti-poly(GA) RAN protein antibody or antigen-binding fragment comprises a light chain variable region (VL) comprising: a CDR1 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23; a CDR2 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 24; and/or a CDR3 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 25.

In some embodiments, the anti-poly(GA) RAN protein antibody or antigen-binding fragment comprises a variable heavy chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 26.

In some embodiments, the anti-poly(GA) RAN protein antibody or antigen-binding fragment comprises a variable light chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 31.

In some embodiments, the anti-poly(GA) RAN protein antibody or antigen-binding fragment comprises a heavy chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 27 or 29.

In some embodiments, the anti-poly(GA) RAN protein antibody or antigen-binding fragment comprises a light chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 32 or 34.

In some embodiments, the anti-poly(GA) RAN protein antigen-binding fragment comprises a single chain variable fragment (scFV) comprising a heavy chain variable region comprising: a CDR1 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 1; a CDR2 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 2; and/or a CDR3 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 3.

In some embodiments, the anti-poly(GA) RAN protein antigen-binding fragment comprises a single chain variable fragment (scFV) comprising a light chain variable region comprising: a CDR1 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 4; a CDR2 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 5; and/or a CDR3 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 6.

In some embodiments, the scFv comprises a variable heavy chain comprising or

consisting of the amino acid sequence set forth in SEQ ID NO: 17.

In some embodiments, the scFv comprises a variable light chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 18.

In some embodiments, the scFv comprises a linker molecule connecting the heavy chain  
5 variable region to the light chain variable region. In some embodiments, the linker molecule comprises a poly-GS linker.

In some embodiments, the antibody or antigen binding fragment thereof further comprises a signal peptide.

In some embodiments, the rAAV vector comprises the sequence set forth in any one of  
10 SEQ ID NOs: 36-40.

In some embodiments, the rAAV vector comprises a sequence that is at least 75% identical to the sequence set forth in any one of SEQ ID NOs: 43-46. In some embodiments, the rAAV vector the rAAV comprises a sequence set forth in any one of SEQ ID NOs: 43-46.

In some aspects, the disclosure provides a recombinant adeno-associated virus (rAAV)  
15 comprising: an rAAV vector as described herein; and one or more adeno-associated virus (AAV) capsid proteins.

In some aspects, the disclosure provides a method for reducing poly(GA) RAN protein aggregation in a subject, the method comprising administering an rAAV as described herein, to the subject.

20 In some embodiments, the one or more AAV capsid proteins comprises an AAV9 capsid protein.

In some embodiments, the one or more AAV capsid proteins comprise an AAV1 capsid protein.

In some embodiments, the one or more AAV capsid proteins comprise a VP1 protein  
25 comprising an ERDRTRG peptide (e.g., as set forth in SEQ ID NO: 49). In some embodiments, the VP1 protein comprises the amino acid sequence set forth in SEQ ID NO: 48.

In some aspects, the disclosure provides a composition comprising an rAAV vector or rAAV as described herein, and a pharmaceutically acceptable carrier or buffer.

In some aspects, the disclosure provides a method for expressing an antibody or antigen-  
30 binding fragment in a subject, the method comprising administering an rAAV as described herein, to the subject.

In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

In some embodiments, the subject expresses one or more RAN proteins. In some  
35 embodiments, the subject expresses poly(GA) RAN protein.

In some embodiments, the subject has or is suspected of having Alzheimer's disease, amyotrophic lateral sclerosis (ALS), or frontotemporal dementia.

In some embodiments, the subject has or is suspected of having ALS.

In some aspects, the disclosure provides a method for treating a subject having ALS, the method comprising administering an rAAV as described herein, to the subject.

In some embodiments, the subject is a human.

#### BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 shows representative immunohistochemistry (IHC) data indicating that anti-poly(GA)-IgG1 antibodies expressed from rAAVs reduce the number of poly(GA) RAN protein aggregates in brain tissue of a C9orf72 mouse model (C9 BAC) of ALS.

FIG. 2 shows representative data indicating that anti-poly(GA)-IgG1 antibodies expressed from rAAVs reduce the number of poly(GA) RAN protein aggregates in a C9orf72 mouse model of ALS. The left-most panel shows a histogram representation of the number (N) of poly(GA) aggregates per nuclei, the middle panel shows a histogram representation of poly(GA) aggregate total area, and the right-most panel shows a histogram representation of average poly(GA) aggregate size. In each histogram, the first bar shows results corresponding to control mice, the second bar shows results corresponding to PBS-treated C9 BAC mice, the third bar shows results corresponding to C9 BAC mice treated with an rAAV9 comprising a transgene encoding anti-poly(GA)-IgG1 A, the fourth bar shows results corresponding to C9 BAC mice treated with an rAAV9 comprising a transgene encoding anti-poly(GA)-ScFv, the fifth bar shows results corresponding C9 BAC mice treated with an rAAV9 comprising a transgene encoding anti-poly(GA)-IgG1 B, and the sixth bar shows results corresponding to C9 BAC mice treated with an rAAV9 comprising a transgene encoding anti-poly(GA)-IgG2 antibodies. N = 5 animals/group. One-way ANOVA with Sidak analyses for multiple comparisons. Data represent mean +/- SEM. \* < 0.05.

FIG. 3 shows representative microscopy data indicating that anti-poly(GA) antibodies administered using AAV-based delivery co-localize with poly(GA) RAN protein aggregates in the brain tissue of C9 BAC mouse models.

FIG. 4 shows representative data indicating AAV9-based delivery of anti-poly(GA) antibodies reduces GP levels in the frontal cortex of C9 BAC mouse models. N ≥ 5 animals/group. In some embodiments, anti-poly(GA) antibodies described herein reduce GP aggregates because they improve protein homeostasis in cells.

FIGs. 5A-5B show representative data indicating AAV9-based delivery of anti-poly(GA) antibodies reduces neuroinflammation in the motor cortex of C9 BAC mouse brains. FIG. 5A

shows immunohistochemistry showing GFAP staining (a marker of neuroinflammation) of motor cortex tissue from non-treated (NT) control mice, PBS-treated C9 BAC mice, and C9 BAC mice treated with rAAV9 comprising a transgene encoding anti-poly(GA) antibodies. FIG. 5B shows quantifications of the data shown in FIG. 5A.  $N \geq 5$  animals/group.

5        FIG. 6 shows representative data indicating a novel monoclonal  $\alpha$ -GA antibody recognizes GA aggregates in C9 BAC mouse brain and a human cell line model of ALS (human patient-derived cells (iMN)).

FIGs. 7A-7B show representative data indicating  $\alpha$ -GA recombinant antibodies are expressed and secreted. FIG. 7A shows western blot analyses of cell protein lysates from  
10    HEK293T cells expressing FLAG-tagged anti-poly(GA)-ScFv or untagged anti-poly(GA)-IgG1 A, anti-poly(GA)-IgG1 B, or anti-poly(GA)-IgG2 antibodies. FIG. 7B shows fluorescence microscopy analyses of supernatant comprising primary antibody (anti-poly(GA) antibodies (FLAG-tagged anti-poly(GA)-ScFv or untagged anti-poly(GA)-IgG1 A, anti-poly(GA)-IgG1 B, or anti-poly(GA)-IgG2 antibodies)) which recognized GA aggregates in HEK293T  
15    overexpressing GFP-GA<sub>60</sub>.

FIG. 8 shows representative data from immunohistochemistry analyses indicating recombinant  $\alpha$ -GA antibodies recognize GA aggregates in C9-mouse brain.

FIGs. 9A-9B show representative data indicating recombinant  $\alpha$ -GA antibodies reduce GFP-GA<sub>60</sub> in HEK293T cells. FIG. 9A shows western blot analyses of protein lysates obtained  
20    from cells expressing  $\alpha$ -GA recombinant antibodies and GFP-GA<sub>60</sub>. FIG. 9B shows a quantification of total GFP-GA levels after treatment with recombinant  $\alpha$ -GA antibodies as shown in FIG. 9A.

FIGs. 10A-10B show representative data obtained from immunofluorescence analyses indicating recombinant  $\alpha$ -GA antibodies reduce GA aggregate number and size. FIG. 10A shows  
25    fluorescence microscopy analyses of protein lysates obtained from cells expressing  $\alpha$ -GA recombinant antibodies and GFP-GA<sub>60</sub>. FIG. 10B shows quantification of the number of aggregates after treatment with recombinant  $\alpha$ -GA antibodies as shown in FIG. 10A. The treatments shown top to bottom in the key correspond to the treatments shown left to right on the x-axis in the histogram.

30        FIGs. 11A-11D show the development of an ERDR-IgG-GFP isotype control which can be delivered using a recombinant AAV. FIG. 11A shows a diagram of a recombinant  $\alpha$ -GA immunoglobulin and a nucleic acid comprising a transgene encoding the recombinant  $\alpha$ -GA immunoglobulin. FIG. 11B shows a diagram of  $\alpha$ -GA single chain variable fragment (scFV) and a nucleic acid comprising a transgene encoding the  $\alpha$ -GA scFV. FIG. 11C shows a diagram of  
35    the  $\alpha$ -GFP isotype control and a nucleic acid comprising a transgene encoding the  $\alpha$ -GFP isotype

control. FIG. 11D shows representative data indicating from fluorescence microscopy analyses of supernatant comprising the  $\alpha$ -GFP isotype control contacted with GFP-transfected cells.

FIG. 12 shows a non-limiting example of an efficacy study strategy using AAV1-ERDR-IgG C9-BAC mice.

5 FIG. 13 shows representative data indicating wide-spread delivery of AAV1-ERDR-TFP to ependymal and motor cortex cells after ICV injection.

FIGs. 14A-14B show representative data obtained from hanging wire tests of injected mouse subjects. FIG. 14A shows hanging wire scores from analyses of injected 16-week-old mouse subjects indicating the number of reaches to either end of the wire and falls. The left  
10 panel represents scores for how many times the animals reached the edge of the apparatus. The middle panel and right panel both represent scores for the number of times the animals fell from the apparatus. In the middle panel, each animal started with a score of 10 and points were deducted based on the number of times the animal fell. In right panel, each animal started with a score of 0 and points were added based on the number of times the animal fell. FIG. 14B shows  
15 photographs taken during hanging wire analyses of injected mouse subjects.

FIGs. 15A-15F show representative data obtained from open-field analyses of injected mouse subjects. FIG. 15A shows open field analysis of C9orf72 positive mice showing variable ambulatory phenotypes compared to non-transgenic littermates. FIG. 15B shows representative data from open field analyses of treated and untreated C9orf72 BAC mice at 23 weeks of age  
20 (ambulatory parameters). FIG. 15C shows representative data from open field analyses of treated and untreated C9orf72 BAC mice at 23 weeks of age (stereotypic behavior, resting time, and exploratory behavior). FIG. 15D shows representative data indicating treatment B rescues 11/12 open field abnormalities in C9-BAC mice compared to vehicle treatment. FIG. 15E shows representative data indicating Group B treated C9-BAC mice show no differences in open field  
25 behavior compared to NT animals. FIG. 15F shows representative data indicating treatment A modestly improves 4 stereotypic grooming/exploration but not ambulation parameters in C9-BAC mice. For FIGs. 15B-15C, treatment groups shown along the x-axis of the plots from left to right are: C9 Uninjected, C9 Vehicle, C9 Apricot, C9 Blue, NT Vehicle, and NT Uninjected. For FIGs. 15D-15F, grey boxes define regions of significant difference found in antibody  
30 treatment cohorts compared to C9-vehicle or NT-vehicle controls ( $p \leq 0.05$ ),  $n \geq 5/\text{group}$ . Data represent mean  $\pm$  SEM. \*  $< 0.05$ , \*\* $< 0.01$ , \*\*\* $< 0.001$ , \*\*\*\* $< 0.0001$ .

FIG. 16 shows representative data indicating treatment B improves survival of C9-BAC mice compared to treatment A and treatment with vehicle. Kaplan-Meier survival curve with the Bonferroni analysis for multiple comparisons between treatment groups at 23 weeks of age. The

analysis showed that C9 mice treated with IgG B significantly increased survival compared to the C9-vehicle and C9 IgGA-treated cohorts.

FIG. 17 shows representative data indicating treatment A reduces GA aggregates in the retrosplenial cortex (RSC) of C9-BAC mouse brains. Compared to C9-vehicle animals, GA

5 RAN aggregates are reduced in the RSC of C9-IgG-B treated mice.

FIG. 18 shows representative data obtained from analyses of the distribution of recombinant  $\alpha$ -GA antibody in brain and spinal cord tissues after AAV-based delivery.

FIGs. 19A-19B show representative data indicating AAV-based delivery of recombinant  $\alpha$ -GA antibody reduces GA aggregates in the frontal cortex of treated C9-BAC mice. FIG. 19A  
10 shows data obtained from immunohistochemistry analyses indicating recombinant  $\alpha$ -GA antibodies recognize GA aggregates in the frontal cortex of treated C9-BAC mice. FIG. 19B shows quantifications of the data in FIG. 19A.

FIGs. 20A-20B show representative data indicating AAV-based delivery of recombinant  $\alpha$ -GA antibody increases motor neuron survival in treated C9-BAC mouse brains. FIG. 20A  
15 shows data obtain from microscopy analyses of treated C9-BAC motor neurons. FIG. 20B shows data obtained from analyses of choline acetyltransferase-positive (ChAT<sup>+</sup>) motor neurons in treated C9-BAC mouse brains.

FIGs. 21A-21B show representative data obtained from microscopy analyses of treated C9-BAC mouse brains indicating recombinant  $\alpha$ -GA antibody is retained in the CA2 region after  
20 AAV-based delivery.

FIGs. 22A-22B show representative data obtained from microscopy analyses of treated C9-BAC mouse brains indicating the biodistribution of recombinant  $\alpha$ -GA antibody in mouse brains after AAV-based delivery.

FIGs. 23A-23B show AAV-GA antibody treatment reduces GA levels in C9 female mice  
25 brain (FIG. 23A) and Organoids (FIG. 23B) derived from C9orf72 ALS/FTD.

FIGs. 24A-24B show AAV-GA antibody improves hanging wire performance (FIG. 24A) and reduces GA levels in the brain of C9-BAC male mice (FIG. 24B).

## 30 DETAILED DESCRIPTION

Aspects of the disclosure relate to compositions and methods for the diagnosis and/or treatment of certain neurodegenerative diseases, for example those diseases associated with repeat-associated non-ATG (RAN) translation proteins, such as ALS. The disclosure is based, in part, on rAAV vectors and rAAV particles encoding antibodies (e.g., monoclonal antibodies)  
35 and antigen-binding fragments thereof (e.g., single-chain variable fragments, scFvs) that bind to

poly-Glycine-Alanine (poly(GA)) RAN proteins. In some embodiments, the disclosure relates to methods of reducing RAN protein (e.g., poly(GA) RAN protein) aggregation in a subject by administering the rAAV vectors or rAAV particles to the subject. In some embodiments, the disclosure relates to methods of treating a RAN protein-associated disease by administering to a  
5 subject in need thereof the rAAVs.

#### *Recombinant AAVs (rAAVs)*

Aspects of the disclosure relate to certain viral vectors, for example, recombinant adeno-associated virus (rAAV) vectors, and rAAV particles (also referred to as “rAAVs”) comprising  
10 such vectors, that express a transgene encoding an anti-RAN protein antibody (e.g., an anti-poly(GA) RAN protein antibody or antigen-binding fragment thereof). In some embodiments, the rAAVs are administered to a subject (e.g., a subject having a disease characterized by translation and accumulation of RAN proteins, such as wherein the rAAVs are administered to treat a human subject having or suspected of having a disease or disorder associated with  
15 translation and accumulation of RAN proteins), and expression of the antibody or antigen-binding fragment thereof reduces RAN protein translation and RAN protein aggregation in the subject.

In some embodiments, a recombinant rAAV particle comprises a nucleic acid vector, such as a single-stranded (ss) or self-complementary (sc) recombinant AAV vector. In some  
20 embodiments, the rAAV vector comprises a transgene encoding an anti-poly(GA) RAN protein antibody or antigen-binding fragment thereof (e.g., scFv) as described herein, and one or more regions comprising inverted terminal repeat (ITR) sequences (e.g., wild-type ITR sequences or engineered ITR sequences) flanking the expression construct. In some embodiments, the rAAV vector is encapsidated by a viral capsid. In some embodiments, the transgene is operably linked  
25 to a promoter, for example a constitutive promoter or an inducible promoter. In some embodiments, the promoter is a tissue-specific (e.g., CNS-specific) promoter.

Accordingly, in some embodiments, a rAAV particle comprises a viral capsid and a nucleic acid vector (e.g., rAAV vector) as described herein, which is encapsidated by the viral capsid. In some embodiments, the viral capsid comprises 60 capsid protein subunits comprising  
30 VP1, VP2 and VP3. In some embodiments, the VP1, VP2, and VP3 subunits are present in the capsid at a ratio of approximately 1:1:10, respectively.

The ITR sequences of a nucleic acid or nucleic acid vector described herein can be derived from any AAV serotype (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) or can be derived from more than one serotype. In some embodiments of the nucleic acid or nucleic acid vector provided  
35 herein, the ITR sequences are derived from AAV2. ITR sequences and plasmids containing ITR

sequences are known in the art and commercially available (see, *e.g.*, products and services available from Vector Biolabs, Philadelphia, PA; Cellbiolabs, San Diego, CA; Agilent Technologies, Santa Clara, Ca; and Addgene, Cambridge, MA; and Gene delivery to skeletal muscle results in sustained expression and systemic delivery of a therapeutic protein. Kessler PD, Podsakoff GM, Chen X, McQuiston SA, Colosi PC, Matelis LA, Kurtzman GJ, Byrne BJ. Proc Natl Acad Sci U S A. 1996 Nov 26;93(24):14082-7; and Curtis A. Machida. Methods in Molecular Medicine™. Viral Vectors for Gene Therapy Methods and Protocols. 10.1385/1-59259-304-6:201 © Humana Press Inc. 2003. Chapter 10. Targeted Integration by Adeno-Associated Virus. Matthew D. Weitzman, Samuel M. Young Jr., Toni Cathomen and Richard Jude Samulski; U.S. Pat. Nos. 5,139,941 and 5,962,313, all of which are incorporated herein by reference). An exemplary AAV2 ITR sequence is shown below.

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTC  
GCCCGACGCCCCGGGCTTTGCCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA  
GGGAGTGGCCAACTCCATCACTAGGGGTTTCCT (SEQ ID NO: 42)

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In some embodiments, the expression construct is no more than 7 kilobases, no more than 6 kilobases, no more than 5 kilobases, no more than 4 kilobases, or no more than 3 kilobases in size. In some embodiments, the expression construct is between 4 and 7 kilobases in size.

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In some embodiments, an rAAV particle comprises a nucleic acid (*e.g.*, a vector or a recombinant genome or transgene thereof) described herein. In some embodiments, an rAAV particle comprises a nucleic acid that is at least 75% identical to any one of SEQ ID NOs: 43-46. For example, in some embodiments, an rAAV particle comprises a nucleic acid that is at least 75% identical to any one of the sequences set forth in SEQ ID NOs: 43-46 such that the nucleic acid comprises ITRs and/or a regulatory sequence(s) that differs relative to the sequence and/or wherein the nucleic acid comprises one or more codons that differ relative the sequence (*e.g.* wherein the nucleic acid is codon optimized). In some embodiments, an rAAV particle comprises a nucleic acid that is 75-80%, 80-85%, 85-90%, 90-95%, 96%, 97%, 98%, or 99% identical to any one of SEQ ID NOs: 43-46. In some embodiments, an rAAV particles comprises a nucleic acid set forth in any one of SEQ ID NOs: 43-46 or a portion thereof, such as the ITRs, the transgene(s), regulatory sequence(s), etc., or any combination thereof. In some embodiments, an anti-GA antibody described herein is encoded by a nucleotide sequence comprised in any one of SEQ ID NOs: 43-46. Non-limiting embodiments of nucleic acids of the disclosure are set forth in Table 1 below.

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Table 1. Representative Vectors for Anti-GA Antibody Expression

| Name                | Nucleic Acid Sequence (5' to 3')                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | SEQ ID NO |
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| pKAAV.ScFv<br>27B11 | cctgcaggcagctgcgcgctcgctcgctcactgagccgcccgggcaaag<br>cccgggcgctcgggcgacctttggtcgcccggcctcagtgagcgagcgagc<br>gcgcagagagggagtgccaaactccatcactaggggttcctacgcgtgtc<br>tgtctgcacatttcgtagagcgagtggtccgatactctaactcctcctagg<br>actagttattaatagtaatacaattacggggtcattagttcatagcccata<br>tatggagttccgcgttacataaacttacggtaaatggccgcctggctgac<br>cgcccaacgacccccgcccattgacgtcaataatgacgtatgttcccata<br>gtaacgccaatagggactttccattgacgtcaatgggtggagtatttacg<br>gtaaaactgcccacttggcagtagcatcaagtgtatcatatgccaaagtacgc<br>cccctattgacgtcaatgacggtaaatggccgcctggcattatgccag<br>tacatgaccttatgggactttcctacttggcagtagcatctacgtattagt<br>catcgctattaccatggtcgaggtgagccccacgttctgcttactctcc<br>ccatctccccccccctccccaccccccaattttgtatttatttttttaa<br>ttattttgtgcagcgatgggggcggggggggggggggggcgcgccagg<br>cggggcggggcggggcgaggggcggggcgggggcgagggcgagaggtgcg<br>cggcagccaatcagagcggcgcgtccgaaagtctccttttatggcgagg<br>cggcggcgcgcgccctataaaaaagcgaagcgcgcgcgcgcgggaggt<br>cgctgcgcgctgccttcgccccgtgccccgctccgccgcgcctcgcgcc<br>gcccgcggcgctctgactgaccgcgttactcccacaggtgagcgggcg<br>gacggcccttctcctccgggctgtaattagcgcttggtttaatgacggct<br>tgtttctttctgtggtgcgtgaaagccttgaggggctccgggagggcc<br>ctttgtgcgggggagcggtcggggggtgcgtgcgtgtgtgtgcgtg<br>gggagcgccgcgtgcggctccgcgctgcccggcggtgtgagcgctgcg<br>gcgcggcgcggggctttgtgcgtccgcagtggtgcgcgaggggagcgcg<br>ccgggggcggtgccccgcggtgcggggggggctgcgaggggaacaaaggc<br>tgcggtgcggggtgtgtgcgtgggggggtgagcagggggtgtggcgcgct<br>ggtcgggctgcaacccccctgcacccccctccccgagttgctgagcacg<br>gcccggcttcgggtgcggggtccgtacggggcggtggcgcggggctcgcc<br>gtgccgggcggggggtggcggcagtgggggtgccgggcggggcggggc<br>gcctcgggccggggagggctcgggggaggggcgcgcgggcccccgagcg<br>ccggcggtgtgcgagggcgggcgagccgcagccattgccttttatggtaa<br>tcgtgcgagagggcgagggacttcccttgcctccaaatctgtgcggagcc<br>gaaatctgggagggcgccgcgcacccccctctagcgggcgcgggggaagc<br>ggtgcggcgccggcaggaaggaatggcggggagggccttcgtgcgtcg<br>ccgcgcgcgctccccttctccctctccagcctcgggggtgtccgcggg<br>ggacggctgccttcgggggggacggggcagggcggggttcggcttctggc<br>gtgtgaccggcggtctagagcctctgctaaccatgttcatgccttcttc<br>tttttctacagctcctgggcaacgtgctggttatgtgctgtctcatca<br>ttttggcaagaattaaaCtcgagggccacgATGGATTGGACTTGGAGAGT<br>GTTTGGCTGCTGGCTGTGCGACCTGGGGCTCATAGTGAGGTGAGCTGC<br>AGGAGTCTGGGGGAGGCTCAGTGCAGCCTGGAGGGTCCCTGAACTCTCC<br>TGCGCAGCCTCTGGATTGCTTTTCTAGTAATATGGCATGTCTTGGGTTTCG<br>CCAGACTCCAGACAAGAGGCTGGAGTTGGTCACAACCATTAATAGTGATG<br>GTGATAGTACCTTTTATCCAGACAGTGTGAAGGGCCGATTACCATCTCC<br>AGAGACAATGCCAAGAACGCCCTGTACCTGCAAATGAGCAGTCTGAAGTC<br>AGACGACACAGCCATGTATTACTGTGCAAGAGTGGGAGGTAACCTACGACT<br>TTGCTATGGACTACTGGGGTCAGGGAACCTCAGTCATCGTCTCTCAGGT<br>GGAGGCGGTTGAGGCGGAGGTGGCAGCGCGGTGGCGGGTCGGACATTGT<br>GATGTACAGTCTTCCATCTCCCTGGCTGTGTGTCAGCAGGAGATAAGGTCA<br>CTATGAGCTGCAAATCCAGTCAGAGTCTGCTCAACAGTAGGACCCGAAAG<br>AACTACTTGGCTTGGTACCAGCAGAAACAGGGCAGTCTCTAACTACT<br>GATCTACTGGACATCCACTCGGGAATCTGGGGTCCCTGATCGCTTCACAG<br>GCAGTCGATCTGGGACAGATTTTACTCTCACCATCAGCAGTGTGCAGGCT<br>GAAGACCTGGCAGTTTATTACTGCAAGCAATCTTATAATAATCCGTGGAC<br>GTTCCGGTGGAGGCACCAAGCTGGAATAAAAgattataaagatcatgatg<br>gcgattataaagatcatgatattgattataaagatgatgatgataaataa<br>gcGGCCGCtagactcgactgtgccttctagttgccagccatctgttg<br>tttgccttcccccggtgccttcttgccttggaaggtgccactcccact<br>gtccttttctaataaaaatgaggaaattgcatcgcatgtgtgagtaggtg | 43        |

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|              | <p>TGGTCAGTGCCCTCCCCATCCAGCACCAGGACTGGATGAGTGGCAAGGAG<br/> TTCAAATGCAAGGTCAACAACAAAGACCTCCCAGCGCCCATCGAGAGAAC<br/> CATCTCAAAACCCAAAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTGC<br/> CTCCACCAGAAGAAGAGATGACTAAGAAACAGGTCACTCTGACCTGCATG<br/> GTCACAGACTTCATGCCTGAAGACATTACGTGGAGTGGACCAACAACGG<br/> GAAAACAGAGCTAAACTACAAGAACACTGAACCAGTCCTGGACTCTGATG<br/> GTTCTTACTTCATGTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTG<br/> GAAAGAAATAGCTACTCCTGTTTCAAGTGGTCCACGAGGGTCTGCACAATCA<br/> CCACACGACTAAGAGCTTCTCCCGGACTCCGGGTAAAgagaacagactt<br/> tgaatgttgaccttctcaagttggcgggagacgtggagtccaacccctgga<br/> cctATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATCCTGC<br/> TTCCAGCAGTgacattgtgatgtcacagtttccatcctccctggctgtgt<br/> cagcaggagataaggctcactatgagctgcaaaccagtcagagctcgtct<br/> aacagtaggaccgaaagaactacttggcttggtagcagcagaaacagg<br/> gcagctctcctaaactactgatctactggacatccactcggaatctggcg<br/> tccctgatcgcttcacaggcagtcgatctgggacagatttactctcacc<br/> atcagcagtggtgcaggctgaagacctggcagtttattactgcaagcaatc<br/> ttataataatccgtggacgttcgggtggaggcaccagcttgaataaaaaC<br/> GGGCTGATGCTGCACCAACTGTATCgATCTTCCCACCATCCAGTGAGCAG<br/> TTAACATCcGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACCTTACCC<br/> CAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAACGACAAAATG<br/> GCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAAGACAGCACCTACAGC<br/> ATGAGCAGCACCCCTCACGTTGACCAAGGACGAGTATGAACGACATAACAG<br/> CTATACCTGTGAGGCCACTCACAAGACATCAACTTACCCATTGTCAAaA<br/> GCTTCAACAGGAATGAGTGTAGGcGGCCGActagacctcgactgtgcc<br/> ttctagttgccagccatctgttgtttgcccctcccccgctgccttctga<br/> ccctggaaggtgccactcccactgtcctttcctaataaaaatgaggaaatt<br/> gcatcgcatgtgtctgagtaggtgtcattctattctggggggtggggggg<br/> gcaggacagcaagggggaggattgggaagacaatagcaggcatgctgggg<br/> aatctagagatctgtgtgttgggtttttgtgttaggaacccctagtgtgg<br/> agttggccactccctctctgcgcgctcgctcgctcactgaggccggcgga<br/> ccaaaggtcgcccgacgcccgggctttgcccgggcgccctcagtgagcga<br/> gcgagcgcgagctgctgcagg</p> |    |
| pKAAV.IgG1-B | <p>cctgcaggcagctgcgcgctcgctcgctcactgaggccgcccgggcaaaag<br/> cccgggctcgggcgaccttggctcgccggcctcagtgagcgagcgagc<br/> gcgcagagagggagtgccaaactccatcactaggggttccctacgcgtgtc<br/> tgtctgcacatttctgtagagcgagtggtccgatactctaattctccctagg<br/> actagttattaatagtaatacaattacggggtcattagttcatagcccata<br/> tatggagttccgcgttacataacttacggtaaatggcccgcctggctgac<br/> cgcccaacgaccccccgccattgacgtcaataatgacgtatgttcccata<br/> gtaacgccaatagggaactttccattgacgtcaatgggtggagattttacg<br/> gtaaaactgcccacttggcagtagatcaagtgtatcatatgccaagtacgc<br/> cccctattgacgtcaatgacggtaaatggcccgcctggcattatgccag<br/> tacatgaccttatgggactttcctacttggcagtagatctacgtattagt<br/> catcgctattaccatggtcgaggtgagccccacgttctgcttactctcc<br/> ccatctccccccctccccaccccccaattttgtatttatttttttaa<br/> ttatttttgtgcagcgatggggggcgggggggggggggggcgcgccagc<br/> cgggggcgggcgggcgagggggcgggggcgggggcgagggcgagaggtgcgg<br/> cggcagccaatcagagcgggcgcgctccgaaagtctccttttatggcgagg<br/> cgggcgggcgggcgggccctataaaaaagcgaagcgcgggcgggcgggaggt<br/> cgctgcgcgctgcttgcggcgctgcccgcctccgcccgcgctcgcgcc<br/> gcccggcccggtctgactgaccgcgttactcccacaggtgagcgggcg<br/> gacggcccttctcctccgggtgtaattagcgcttggtttaatgacggct<br/> tgtttctttctgtggtgctgaaagccttgaggggctccgggagggcc<br/> ctttgtgcggggggagcggtcggggggtgcgtgcgtgtgtgtgtgcgtg<br/> gggagcgccgctgcggctccgcgctgcccggcggtgtgagcgctgcgg<br/> gcgcggcgggggctttgtgcgtccgcagtggtgcgcgaggggagcgcg<br/> ccggggcggtgccccgcggtgcggggggggctgcgaggggaacaaaggc<br/> tgcggtgcgggggtgtgtgcgtgggggggtgagcagggggtgtggcgcgct<br/> ggctgggctgcaacccccctgcacccccctcccaggttgctgagcagc<br/> gcccggcttcgggtgcggggctccgtacggggcggtggcgcggggtcgcc<br/> gtgcggggcggggggtggcggcaggtgggggtgcccggcgggggcgggggc<br/> gcctcgggcgggggagggctcgggggagggggcgcgcgggcccccgagcg</p>                                                                                                                                                  | 45 |

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| pKAAV.IgG1-A<br>27B11 | cctgcaggcagctgcgcgctcgctcgctcactgagggcgcccgggcacaag<br>cccgggcgctcgggcgacctttgggtcgccggcctcagtgagcgagcgagc<br>gcgcagagagggagtgccaaactccatcactaggggttcctacgcgtgtc<br>tgtctgcacatttcgtagagcgagtggtccgatactctaactctccctagg<br>actagttattaatagtaatacaattacggggtcattagttcatagcccata<br>tatggagttcccggttacataacttacggtaaatggcccgctggctgac<br>cgcccaacgacccccgccattgacgtcaataatgacgtatgttcccata<br>gtaacgccaatagggaactttccattgacgtcaatgggtggagttttacg<br>gtaaactgcccaacttggcagtagatcaagtgtatcatatgccaagtacgc<br>cccctattgacgtcaatgacggtaaatggcccgctggcattatgccag<br>tacatgaccttatgggactttcctacttggcagtagatctacgtattagt<br>catcgctattaccatgggtcgaggtgagccccacgttctgcttcactctcc<br>ccatctccccccctccccaccccccaattttgtatttatttatttttaa<br>ttattttgtgcagcgtggggcgggggggggggggggcgcgcgagcag<br>cgggcgggcgggcgggcgagggcgggcgggcgagggcgagaggtgagg<br>cggcagccaatcagagcgggcgcgctccgaaagtttccttttatggcgagg<br>cggcggcgggcgggcgccctataaaaaagcgaagcgcgcgggcggggag<br>cgctgcgcgctgccttcgccccgtgccccgctccgccgcgcctcgcgcc<br>gcccggcccgctctgactgaccggttaactcccacaggtgagcgggcg<br>gacggcccttctcctccgggtgtaattagcgcttggtttaatgacggct<br>tgtttctttctgtggctgcgtgaaagccttgaggggtccgggagggcc<br>ctttgtgcgggggagcggtcggggggtgcgtgcgtgtgtgtgtgcgtg<br>gggagcgccgctgcggctccgcgctgccggcggtgtgagcgctgcgg<br>gcgcggcgcggggtttgtgcgtccgcagtggtgcgcgaggggagcgcg<br>ccggggcggtgcccccggtgcgggggggggtgcgaggggaacaaaggc<br>tgctgcgggggtgtgtgcgtgggggggtgagcaggggtgtggcgcgct<br>gggtcggtgcacccccctgcacccccctccccaggtgtgtagcacg<br>gcccggcttcgggtgcgggtccgtacggggcggtggcgcggggtcgcc<br>gtgcccggcggggggtggcggcaggtgggggtgcccggcgggcgggggcc<br>gcctcgggccggggagggctcgggggaggggagcgcgggcccccgagcg<br>ccggcggtgtgcagcgcgggcgagcgcgagccattgccttttatggtaa<br>tcgtgcgagagggcgagggacttcctttgtcccaaatctgtgcggagcc<br>gaaatctgggagggcgccgcgcaccccccttagcgggcgggggcgagc<br>ggtgcggcgccggcaggaaggaaatggcggggagggccttcgtgcgtcg<br>ccgcgcgcgcgtcccccttctccctctccagcctcggggtgtcccgggg<br>ggacggctgccttcgggggggacggggcagggcggggttcggcttctggc<br>gtgtgaccggcggtctagagcctctgctaaccatgttcatgccttcttc<br>tttttctacagctcctgggcaacgtgctggttattgtgctgtctcatca<br>ttttggcaagaattaaaCtcgagggcacgatggattggacttgagagt<br>gttttgctgctggctgtcgacctggagctcatagtgaggtgagctgc<br>aggagtctggggaggtcagtgacgctggaggggtccctgaaactctcc<br>tgcgagcctctggattcgctttcagtaactatggcatgtcttgggttcg<br>ccagactccagacaagaggtggagttggtcacaccattaatagtgatg<br>gtgatagtagcttttatccagacagtggaagggccgattaccatctcc<br>agagacaatgccaaagacgcctgtacctgcaaatgagcagctctgaagtc<br>agacgacacagccatgtattactgtgcaagagtgggaggttaactacgact<br>ttgctatggactactgggtcagggaaacctcagtcacgtGtcctcaGCT<br>AAAACGACACCCCATCTGTCTATCCACTGGCCCTGGATCTGTGCCA<br>AACTAACTCgATGGTGACCTGGGATGCCTGGTCAAGGGCTATTTCCTG<br>AGCCAGTGACAGTGACCTGGAACCTCTGGTTCCTGTCCAGCGGTGTGCAC<br>ACCTTCCAGCTGTCTCTCAGTCTGACCTCTACACTCTGAGCAGCTCAGT<br>GACTGTCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTG<br>CCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCAGGGAT<br>TGTGGTTGTAAGCCTTGCAATAGcACAGTCCCAGAAGTATCATCTGTCTT<br>CATCTTCCCCCAAAGCCCAAGGATGTGCTCACCATTACTCTGACTCCTA<br>AGGTACAGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAG<br>TTCAGCTGGTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACC<br>CCGGGAGGAGCAGTTCAACAGCACTTCCGCTCAGTCAGTGAACCTCCCA<br>TCATGCACCAGGACTGGCTCAATGGCAAGGAGTTCAAATGCAGGGTCAAC<br>AGTGCAGCTTCCCTGCCCCATCGAGAAAACCATCTCCAAAACCAAGG<br>CAGACCGAAGGCTCCGCAGGTcTACACCATTCACCTCCCAAGGAGCAGA<br>TGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTCCT<br>GAAGACATTACTGTGGAGTGGCAGTGAATGGGCAGCCAGCGGAGAATA | 46 |
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|  | CAAGAACACTCAGCCCATCATGGACACAGATGGCTCTTACTTCGTCTACA<br>GCAAGCTCAATGTGCAGAAGAGCAACTGGGAGGCAGGAAATACTTTCACC<br>TGCTCTGTGTTACATGAGGGCCTGCACAACCACCATACTGAGAAGAGCCT<br>CTCCCACTCTCCTGGTAAAgtaaagagactttgaattttgaccttctca<br>agttggcgggaGACGTCgagtcacacccctggacccATGAAGTTGCCTGTT<br>AGGCTGTTGGTGCTGATGTTCTGGATCCCTGCTTCCAGCAGTGACATTGT<br>GATGTCACAGTTTCCATCCTCCCTGGCTGTGTGTCAGCAGGAGATAAGGTCA<br>CTATGAGCTGCAATCCAGTCAGAGTCTGCTCAACAGTAGGACCCGAAAG<br>AACTACTTGGCTTGGTAtCAGCAGAAACCAGGGCAGTCTCCTAAACTACT<br>GATCTACTGGACATCCACTCGGGAATCTGGcGTCCCTGATCGCTTCACAG<br>GCAGTCGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTAcAGGCT<br>GAAGACCTGGCAGTTTATTACTGCAAGCAATCTTATAATAATCCGTGGAC<br>GTTTCGGTGGAGGCACCAAGCTtGAAATAAAACGGGCAGATGCTGCacCAA<br>CTGTATCgATCTTCCCACCATCCAGTGAGCAGTTAACATCcGGAGGTGCC<br>TCAGTCGTGTGCTTCTTGAACAACCTTACCCCAAAGACATCAATGTCAA<br>GTGGAAGATTGATGGCAGTGAACGACAAAATGGCGTCTGAACAGTTGGA<br>CTGATCAGGACAGCAAGACAGCACCTACAGCATGAGCAGCACCTCAGC<br>TTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCAC<br>TCACAAGACATCAACTTCACCCATTGTCAAaAGCTTCAACAGGAATGAGT<br>GTTAGgcGGCCGcactagacctcgactgtgccttctagttgccagccatc<br>tgttggttggccctccccctgccttcttgacctggaaggtgccactc<br>ccactgtcctttcctaataaaaatgaggaaattgcacgcattgtctgagt<br>aggtgtcattctattctgggggtgggtggggcaggacagcaaggggga<br>ggattgggaagacaatagcaggcatgctggggaatctagagatctgtgtg<br>ttggtttttgtgtaggaaccctagtgtgagtggtggccactccctctc<br>tgcgcgctcgctcgctcactgaggccgggacgacaaaggtcgcccgacgc<br>ccgggctttgcccgggcgccctcagtgagcgagcgagcgcgagctgcct<br>gcagg |  |
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The capsid of the rAAV particle may be of any AAV serotype (*e.g.*, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or AAV10), including any derivative (including non-naturally occurring variants of a serotype) or pseudotype. In some embodiments, an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or AAV10 capsid protein is of a mammalian AAV serotype (mammalian AAV1, mammalian AAV2, mammalian AAV3, mammalian AAV4, mammalian AAV5, mammalian AAV6, mammalian AAV7, mammalian AAV8, mammalian AAV9, or mammalian AAV10, respectively). In some embodiments, the AAV capsid protein is an AAV9 capsid protein. In some embodiments, the AAV capsid protein is an AAV1 capsid protein.

In some embodiments, an rAAV particle is a pseudotyped rAAV particle. Non-limiting examples of rAAV pseudotypes include mammalian AAV2/1, mammalian AAV2/5, mammalian AAV2/6, mammalian AAV2/8, mammalian AAV2/9, mammalian AAV3/1, mammalian AAV3/5, mammalian AAV3/8, and mammalian AAV 3/9, wherein the slash denotes an rAAV genome of one serotype packaged in the capsid from a different serotype (*e.g.*, an rAAV genome comprising AAV2 ITRs packaged in a capsid of AAV5 would be AAV2/5).

In some embodiments, an rAAV particle comprises a hybrid or mutant mammalian AAV capsid protein derivative, such as AAVrh.10, AAVrh.74, AAVhu.14, AAV3a/3b, AAVrh32.33, AAV-HSC15, AAV-HSC17, AAVhu.37, AAVrh.8, CHt-P6, AAV2.5, AAV6.2, AAV2i8,

AAV-HSC15/17, AAVM41, AAV9.45, AAV6(Y445F/Y731F), AAV2.5T, AAV-HAE1/2, AAV clone 32/83, AAVShHIO, AAV2 (Y->F), AAV8 (Y733F), AAV2.15, AAV2.4, AAVM41, AAV2(pentaYF), AAV2-BCDG(T491V+K556R), AAV5-M2, AAV5(Y719F), AAV6(T492V+S663V), AAV6(T492V+Y705F+Y731F), AAV6(S551V+S663V), AAV8-  
 5 C&G(T494V), AAV8-M3, AAV8(Y733F), AAV8(T494V+Y733F), AAV8(Y275F+Y447F+Y733F), AAV9-PHP.B, or AAVr3.45.

In some embodiments, a VP1 protein of an AAV capsid comprises an ERDRTRG peptide (SEQ ID NO: 49). The VP1 protein comprising the ERDRTRG (SEQ ID NO: 49) peptide can be of any AAV serotype (e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6,  
 10 AAV7, AAV8, AAV9, or AAV10). In some embodiments, the VP1 variant protein is of serotype AAV9. In some embodiments, the VP1 variant protein is of serotype AAV1. In some embodiments, a VP1 protein of serotype AAV1 comprises an amino acid sequence set forth in SEQ ID NO: 48 as shown below.

15 AAV1 Capsid Peptide with ERDRTRG (SEQ ID NO: 49) Peptide Insertion

MAADGYLPDWLEDNLSEGIREWDDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNLDKGEPVNAADAAALEHDK  
 AYDQQLKAGDNFYLRYNHADADEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEQSPQEPD  
 SSSGIGKTGQQPAKKRLNFGQTGDSESVDPQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGVGNASGNWHCD  
 20 STWLGDRTVITSTRTVALPTYNNHLYKQISSASTGASNDNHYFGYSTPWGYFDFNRFHCHFSRDPWQRLINNNGWFR  
 PKRLNFKLFNIQVKEVTNDGVTTIANNLSTVQVFSDEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGS  
 QAVGRSSFYCLEYFPSQMLRTGNFTFSYTFEEVPFHSSYAHSQSLDRLMNPLIDQYLYLNRTQNQSGSAQNKDLL  
 FSRGSPAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSFTWTGASKYNLNGRESIINPGTAMASHKDDDEDKFFPMSG  
 VMIFGKESAGASNTALDNVMITDEEEIKATNPVATERFGTVAVNFQSSSTDSSAERDRTRGASPATGADVHAMGALPG  
 25 MVWQDRDVYLGPIWAKIPHTDGHFHPSPLMGGFGLKNPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSV  
 EIEWELQKENSKRWNPEVQYTSNYAKSANVDFTVDDNNGLYTEPRPIGTRYLTRPL\* (SEQ ID NO: 48)

Additional AAV serotypes and derivatives/pseudotypes, and methods of producing such derivatives/pseudotypes are known in the art (see, e.g., Mol Ther. 2012 Apr;20(4):699-708. doi:  
 30 10.1038/mt.2011.287. Epub 2012 Jan 24. The AAV vector toolkit: poised at the clinical crossroads. Asokan A1, Schaffer DV, Samulski RJ.).

Generally, rAAV particles are produced in host cells that have been contacted with a helper nucleic acid and a packaging nucleic acid which support AAV replication. Such functions include, without limitation, activation of AAV gene transcription, stage specific AAV mRNA  
 35 splicing, AAV DNA replication, synthesis of capsid expression products, and AAV capsid assembly.

A helper nucleic acid will typically comprise an E1 gene, an E2A gene, an E4 gene, a VA gene, or any combination thereof. In some embodiments, a helper nucleic acid is provided by contacting a host cell with a vector (e.g., a plasmid) comprising a helper nucleic acid. In  
 40 some embodiments, a helper nucleic acid is provided by contacting a host cell with a helper

virus. In some embodiments, a helper virus is an adenovirus (e.g., human adenovirus 2, human adenovirus 5, or human adenovirus 12) or a recombinant herpes simplex virus comprising AAV helper genes. Preferably, the AAV helper nucleic acid supports efficient rAAV particle production without generating any detectable wild-type AAV particles (e.g., AAV particles containing functional rep and capsid protein genes). Helper nucleic acids, and methods of making said nucleic acids, have been previously described and are commercially available (e.g., see the following helper nucleic acids and references which are incorporated by reference for disclosures related to rAAV production: pDM, pDG, pDP1rs, pDP2rs, pDP3rs, pDP4rs, pDP5rs, pDP6rs, pDG(R484E/R585E, and pDP8.ape plasmids from PlasmidFactory, Bielefeld, Germany; other products and services available from Vector Biolabs, Philadelphia, PA; Cellbiolabs, San Diego, CA; Agilent Technologies, Santa Clara, Ca; and Addgene, Cambridge, MA; pxx6; Grimm et al. (1998), Novel Tools for Production and Purification of Recombinant Adeno associated Virus Vectors, Human Gene Therapy, Vol. 9, 2745-2760; Kern, A. et al. (2003), Identification of a Heparin-Binding Motif on Adeno-Associated Virus Type 2 Capsids, Journal of Virology, Vol. 77, 11072-11081.; Grimm et al. (2003), Helper Virus-Free, Optically Controllable, and Two-Plasmid-Based Production of Adeno-associated Virus Vectors of Serotypes 1 to 6, Molecular Therapy, Vol. 7, 839-850; Kronenberg et al. (2005), A Conformational Change in the Adeno-Associated Virus Type 2 Capsid Leads to the Exposure of Hidden VP1 N Termini, Journal of Virology, Vol. 79, 5296-5303; and Moullier, P. and Snyder, R.O. (2008), International efforts for recombinant adeno associated viral vector reference standards, Molecular Therapy, Vol. 16, 1185-1188).

A packaging nucleic acid typically provides nucleotide sequences, including sequences encoding AAV rep protein and AAV capsid proteins, upon which an AAV is dependent for replication. In some embodiments, an rAAV described herein is produced using a packaging nucleic acid comprising a nucleotide sequence encoding an AAV capsid protein of a mammalian AAV serotype. In some embodiments, an rAAV described herein is produced using a packaging nucleic acid comprising the nucleotide sequence of GAGAGGGATCGGACTAGGGGT (SEQ ID NO: 50) which is located in a sequence encoding an AAV capsid protein. In some embodiments, an rAAV comprising a VP1 variant protein comprising an ERDRTRG (SEQ ID NO: 49) peptide as described herein is produced using a packaging nucleic acid comprising the nucleotide sequence of SEQ ID NO: 47 as shown below. In some embodiments, the nucleotide sequence encoding the ERDRTGR (SEQ ID NO: 49) peptide is codon-optimized.

Nucleic acid sequence encoding AAV1 capsid protein with ERDRTRG (SEQ ID NO: 49) peptide insertion

ATGGCTGCCGATGGTTATCTTCAGATTGGCTCGAGGACAACCTCTCTGAGGGCATTGCGAGTGGTGGGACTTGAA  
 ACCTGGAGCCCCGAAGCCCCAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTGCTTCCTGGCTACAAGT  
 ACCTCGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGGGGACGACGCGGCCCTCGAGCACGACAAG  
 5 GCCTACGACCAGCAGCTCAAAGCGGGTGACAATCCGTACCTTCGGTATAACCACGCGGACGCGGAGTTTCAGGAGCG  
 TCTGCAAGAAGATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCTC  
 TCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAACGTCCGGTAGAGCAGTCGCCACAAGAGCCAGAC  
 TCCTCCTCGGGCATCGGCAAGACAGGCCAGCAGCCCGTAAAAAGAGACTCAATTTGGTCAGACTGGCGACTCAGA  
 10 GTCAGTCCCCGACCCACAACCTCTCGGAGAACCTCCAGCAACCCCGCTGCTGTGGGACCTACTACAATGGCTTCAG  
 GCGGTGGCGCACCAATGGCAGACAATAACGAAGGCGCCGACGGAGTGGGTAATGCCTCAGGAAATTGGCATTGCGAT  
 TCCACATGGCTGGGCGACAGAGTCATCACCACCAGCACCCGACCTGGGCCTTGCCACCTACATAACCACCTCTA  
 CAAGCAAATCTCCAGTGCTTCAACGGGGGCCAGCAACGACAACCACTACTTCCGGCTACAGCACCCCTGGGGGTATT  
 TTGATTTCAACAGATTCCACTGCCACTTTTACCACGTGACTGGCAGCGACTCATCAACAACAATTGGGGATTCCGG  
 CCAAGAGACTCAACTTCAAACTCTTCAACATCCAAGTCAAGGAGGTCACGACGAATGATGGCGTCACAACCATCGC  
 15 TAATAACCTTACCAGCACGGTTCAAGTCTTCTCGGACTCGGAGTACCAGCTTCCGTACGTCTCGGCTCTGCGCACC  
 AGGGCTGCCCTCCCTCCGTTCGGGGCGGACGTGTTTATGATTCCGCAATACGGCTACCTGACGCTCAACAATGGCAGC  
 CAAGCCGTGGGACGTTTCATCTTTTACTGCCTGGAATATTTCCCTTCTCAGATGCTGAGAACGGGCAACAACCTTTAC  
 CTTCAGCTACACCTTTGAGGAAGTGCCTTTCCACAGCAGCTACGCGCACAGCCAGAGCCTGGACCGGCTGATGAATC  
 CTCTCATCGACCAATACCTGTATTACCTGAACAGAACTCAAAATCAGTCCGGAAGTGCCCAAAACAAGGACTTGCTG  
 20 TTAGCCGTGGGTCTCCAGCTGGCATGTCTGTTTCAGCCCAAAAAGTGGCTACCTGGACCTGTTATCGGCAGCAGCG  
 CGTTTCTAAAAACAAAACAGACAACAACAACAGCAATTTTACCTGGACTGGTGCTTCAAAATATAACCTCAATGGGC  
 GTGAATCCATCATCAACCTGGCACTGCTATGGCCTCACACAAGACGACGAAGACAAGTTCTTTCCCATGAGCGGT  
 GTCATGATTTTGGAAAAGAGAGCGCCGAGCTTCAACACTGCATTGGACAATGTCATGATTACAGACGAAGAGGA  
 AATTAAAGCCACTAACCTGTGGCCACCGAAAGATTGGGACCGTGGCAGTGAATTTCCAGAGCAGTTCGACGGACT  
 25 CCTCAGCAGAGAGGGATCGGACTAGGGTCTAGCCCTGCCACTGGTGACGTGCATGCTATGGGTGCCTTACCTGGC  
 ATGGTGTGGCAAGATAGAGACGTGTACCTGCAGGGTCCCATTTGGGCCAAAATTCTCACACAGATGGACACTTTCA  
 CCCGTCTCCTTATGGGCGGCTTTGGACTCAAGAACCCGCTCCTCAGATCCTCATAAAAACAGCCTGTTCTCTG  
 CGAATCCTCCGGCGGAGTTTTCAGCTACAAAGTTTGCTTCATTTCATACCCAATACTCCACAGGACAAGTGAGTGTG  
 GAAATTGAATGGGAGCTGCAGAAAGAAAACAGCAAGCGCTGGAATCCCGAAGTGACGTACACATCCAATTATGCAAA  
 30 ATCTGCCAACGTTGATTTTACTGTGGACAACAATGGACTTTATACTGAGCCTCGCCCCATTGGCACCCGTTACCTTA  
 CCGTCCCCGTGTA (SEQ ID NO: 47)

In some embodiments, the components cultured in a host cell to package a rAAV  
 genome in a capsid may be provided to the host cell in trans. In some embodiments, rAAV  
 35 particles may be produced using the triple transfection method (see, e.g., U.S. Pat. No.  
 6,001,650 which is incorporated by reference herein for its disclosures related to triple  
 transfection methods for rAAV production). Typically, the rAAV particles are produced by  
 transfecting a host cell with an AAV vector (see, e.g., Table 1) to be packaged into rAAV  
 particles, and at least one nucleic acid comprising AAV helper genes and/or packaging genes. In  
 40 some embodiments, two nucleic acids are used which include a helper nucleic acid and a packaging  
 nucleic acid. In some embodiments, any one or more of the components used for rAAV particle  
 production (e.g., AAV vector, AAV rep sequences, AAV cap sequences, and/or helper nucleic  
 acids) may be provided by a host cell which has been engineered to stably comprise the one or  
 more components. Such a host cell will typically comprise the one or more components under  
 45 the control of either an inducible promoter or a constitutive promoter. Examples of suitable  
 inducible and constitutive promoters. Methods used to construct the nucleic acids or rAAV  
 particles thereof have been previously described (see, e.g., Sambrook et al., Molecular Cloning:  
 A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, N.Y., which are  
 incorporated by reference for disclosures related to nucleic acid engineering, also see K. Fisher

et al., J. Virol., 70:520-532 (1993) and U.S. Pat. No. 5,478,745, which are incorporated by reference for disclosures related to methods of generating rAAVs).

5 *RAN Proteins*

A “RAN protein (repeat-associated non-ATG translated protein)” is a polypeptide that is translated from sense or antisense RNA sequences bidirectionally transcribed from a repeat expansion mutation in the absence of an AUG initiation codon. RAN protein-encoding sequences can be found in the genome at multiple loci, including but not limited to open reading  
10 frame 72 of chromosome 9 (C9orf72), open reading frame 80 of chromosome 2 (C2orf80), LRP8, CASP8, CRNDE, EXOC6B, SV2B, PPML1, ADARB2, GREB1, and MSMD1. The protein associated with C9orf72 is currently poorly characterized but known to be abundant in neurons, especially in the cerebral cortex and motor neurons. C9orf72 protein is believed to localized in presynaptic termini. C9orf72 protein likely impacts transcription, translation and  
15 intra-cellular localization of RNA. C9orf72 gene contains a GGGGCC repeat. This hexanucleotide repeat occurs in variable repeat numbers, and small numbers of repeats are not associated with any pathology.

Generally, RAN proteins comprise expansion repeats of a single amino acid, di-amino acid, tri-amino acid, or quad-amino acid (*e.g.*, tetra-amino acid), termed poly amino acid repeats.  
20 For example, “AAAAAAAAAAAAAAAAAAAAA” (SEQ ID NO: 51) (poly-Alanine), “LLLLLLLLLLLLLLLLLLLLL” (SEQ ID NO: 52) (poly-Leucine), “SSSSSSSSSSSSSSSSSSSS” (SEQ ID NO: 53) (poly-Serine), or “CCCCCCCCCCCCCCCCCCCCC” (SEQ ID NO: 54) (poly-Cysteine) are poly amino acid repeats that are each 20 amino acid residues in length. Examples of di-amino acid RAN proteins include GPGPGPGPGPGPGPGPGPGP (SEQ ID NO: 55) (poly-GP), GAGAGAGAGAGAGAGAGAGA (SEQ ID NO: 56) (poly-GA),  
25 GRGRGRGRGRGRGRGRGRGR (SEQ ID NO: 57) (poly-GR), PAPAPAPAPAPAPAPAPAPA (SEQ ID NO: 58) (poly-PA), and PRPRPRPRPRPRPRPRPRPR (SEQ ID NO: 59) (poly-PR). Examples of tetra-amino acid repeats include LPACLPACLPAC (SEQ ID NO: 60) (*e.g.*, poly-LPAC) and  
30 QAGRQAGRQAGR (SEQ ID NO: 61) (*e.g.*, poly-QAGR). RAN proteins can have a poly amino acid repeat of at least 20, at least 25, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, or at least 200 amino acid residues in length. In some embodiments, a RAN protein has a poly amino acid repeat more than 200 amino acid residues (*e.g.*, 500, 1000, 5000, 10,000, etc.) in length.

Generally, RAN proteins are translated from abnormal repeat expansions (*e.g.*, TCT repeats, hexanucleotide repeats such as GGGGCC, *etc.*) of DNA. The disclosure is based, in part, on the identification of microsatellite repeats in certain subjects having a RAN protein-associated disease that is characterized by expression of one or more (*e.g.*, 2, 3, 4, 5, or more) RAN proteins, for example poly(Glycine-Alanine) [poly(GA)]. In some embodiments, the disease status of a subject having or suspected of having a RAN protein-associated disease is classified by the number and/or type of microsatellite repeats present (*e.g.*, detected) in the subject (*e.g.*, in the genome of a subject or in a gene of the subject).

A “subject having or suspected of having a disease (*e.g.*, neurological diseases) associated with RAN protein expression, translation, and/or accumulation” generally refers to a subject exhibiting one or more signs and symptoms of a neurodegenerative disease, including but not limited to memory deficit (*e.g.*, short term memory loss), confusion, deficiencies of executive functions (*e.g.*, attention, planning, flexibility, abstract thinking, *etc.*), loss of speech, degeneration or loss of motor skills, *etc.*, or a subject having or being identified as having one or more genetic mutations associated with RAN protein expression, translation, and/or accumulation.

A “subject having or suspected of having amyotrophic lateral sclerosis (ALS)” can be a subject exhibiting one or more signs and symptoms of ALS, including but not limited to memory deficit (*e.g.*, short term memory loss), confusion, deficiencies of executive functions (*e.g.*, attention, planning, flexibility, abstract thinking, *etc.*), loss of speech, degeneration or loss of motor skills, *etc.*, or a subject having or being identified as having one or more genetic mutations associated with ALS, for example mutations in specific genes including C9orf72. In some embodiments, a subject has been diagnosed as having ALS by a medical professional. A “subject having or suspected of having Alzheimer’s disease (AD)” can be a subject exhibiting one or more signs and symptoms of AD, including but not limited to memory deficit (*e.g.*, short term memory loss), confusion, deficiencies of executive functions (*e.g.*, attention, planning, flexibility, abstract thinking, *etc.*), loss of speech, degeneration or loss of motor skills, *etc.*, or a subject having or being identified as having one or more genetic mutations associated with AD, for example mutations in specific genes. In some embodiments, a subject has been diagnosed as having AD by a medical professional. A “subject having or suspected of having frontotemporal dementia (FTD)” can be a subject exhibiting one or more signs and symptoms of FTD, including but not limited to memory deficit (*e.g.*, short term memory loss), confusion, deficiencies of executive functions (*e.g.*, attention, planning, flexibility, abstract thinking, *etc.*), loss of speech, degeneration or loss of motor skills, *etc.*, or a subject having or being identified as having one or more genetic mutations associated with FTD, for example mutations in specific genes. In some

embodiments, a subject has been diagnosed as having ALS by a medical professional. A subject can be a mammal (*e.g.*, human, mouse, rat, dog, cat, or pig). In some embodiments, a subject is a non-human animal, for example a mouse, rat, guinea pig, cat dog, horse, camel, etc. In some embodiments, the subject is a human.

5 In some embodiments, a subject having less than 10 repeat sequences does not exhibit signs or symptoms of a RAN protein-associated disease characterized by RAN protein translation. In some embodiments, a subject having between 10 and 40 repeats may or may not exhibit one or more signs or symptoms of a RAN protein-associated disease characterized by RAN protein translation. In some embodiments, a subject having more than 40 trinucleotide  
10 repeats exhibits one or more signs or symptoms of a RAN protein-associated disease characterized by RAN protein translation. In certain cases, a subject is identified as having a RAN protein-associated disease characterized by large (>100) number of repeats. Microsatellite repeat sequences encoding RAN proteins are generally known. In some embodiments, the RAN protein-associated disease is Alzheimer's disease.

15 In some embodiments, a subject having or suspected of having a RAN protein-associated disease has one or more microsatellite repeat sequences encoding a poly(GA) RAN protein. Examples of microsatellite repeat sequences encoding poly(GA) proteins include GGGGCC.

In some aspects, the disclosure relates to the discovery that RAN protein (*e.g.*, poly(GA), etc.) aggregation patterns are length-dependent. For example, RAN proteins having poly amino  
20 acid repeats that are >20, >48, or >80 residues in length aggregate differently in the brain of a subject. Generally, the differential aggregation properties of RAN proteins having different lengths can be used to detect RAN proteins in a biological sample. Longer RAN proteins are found at higher levels in biological samples, such as blood, serum, or CSF. In some  
25 acid residues in length are detectable in a biological sample.

### *Monoclonal Antibodies*

Various aspects of the disclosure relate to antibodies and antigen-binding fragments that specifically bind to RAN proteins, and methods of making and using the antibodies and antigen-  
30 binding fragments. In some embodiments, the antibody or antigen binding fragment specifically binds to a poly(glycine-alanine) [poly(GA)] RAN protein. In some embodiments, the antibody binds directly to the poly(GA) repeat region (*e.g.*, binds to the GAGAGA (SEQ ID NO: 62) motif). In some embodiments, the antibody binds to a region of the RAN protein (*e.g.*, poly(GA) RAN protein) that is not the repeat region, for example a unique C-terminal amino acid sequence  
35 of the RAN protein.

An antibody, as used herein, broadly refers to an immunoglobulin molecule or any functional mutant, variant, or derivation thereof. It is desired that functional mutants, variants, and derivations thereof, as well as antigen-binding fragments, retain the essential epitope binding features of an Ig molecule. Antibodies are capable of specific binding to a target through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. Generally, an intact or full-length antibody comprises two heavy chains and two light chains. Each heavy chain contains a heavy chain variable region (VH) and a first, second and third constant regions (CH1, CH2 and CH3). Each light chain contains a light chain variable region (VL) and a constant region (CL). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). CDR constituents on the heavy chain are referred to as CDRH1, CDRH2, and CDRH3, while CDR constituents on the light chain are referred to as CDRL1, CDRL2, and CDRL3.

The CDRs typically refer to the Kabat CDRs, as described in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services (1991), eds. Kabat et al. Another standard for characterizing the antigen binding site is to refer to the hypervariable loops as described by Chothia. *See, e.g.,* Chothia, D. et al. (1992) J. Mol. Biol. 227:799-817; and Tomlinson et al. (1995) EMBO J. 14:4628-4638. Still another standard is the AbM definition used by Oxford Molecular's AbM antibody modeling software. *See, generally, e.g.,* Protein Sequence and Structure Analysis of Antibody Variable Domains. In: Antibody Engineering Lab Manual (Ed.: Duebel, S, and Kontermann, R., Springer-Verlag, Heidelberg). Embodiments described with respect to Kabat CDRs can alternatively be implemented using similar described relationships with respect to Chothia hypervariable loops or to the AbM-defined loops, or combinations of any of these methods.

Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDRL1, FR2, CDR2, FR3, CDR3, FR4. A full-length antibody can be an antibody of any class, such as IgD, IgE, IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant domain of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.,* IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term “antigen-binding fragment” refers to any derivative of an antibody which is less than full-length, and that can bind specifically to a target. Preferably, antigen-binding fragments provided herein retain the ability to specifically bind to RAN protein. An antigen-binding fragment may comprise the heavy chain variable region (VH), the light chain variable region (VL), or both. Each of the VH and VL typically contains three complementarity determining regions CDR1, CDR2, and CDR3.

Examples of antigen binding fragments include, but are not limited to, Fab, Fab', F(ab')<sub>2</sub>, scFv, Fv, dsFv, diabody, affibodies, and Fd fragments. Antigen binding fragments may be produced by any appropriate means. For instance, an antigen binding fragment may be enzymatically or chemically produced by fragmentation of an intact antibody or it may be recombinantly produced from a gene encoding the partial antibody sequence. Alternatively, an antigen binding fragment may be wholly or partially synthetically produced. An antigen binding fragment may optionally be a single chain antibody fragment. Alternatively, a fragment may comprise multiple chains which are linked together, for instance, by disulfide linkages. An antigen binding fragment may also optionally be a multimolecular complex. A functional antigen binding fragment will typically comprise at least about 50 amino acids and more typically will comprise at least about 200 amino acids.

Single-chain Fvs (scFvs) are recombinant antigen binding fragments consisting of only the variable light chain (VL) and variable heavy chain (VH) covalently connected to one another by a polypeptide linker. Either VL or VH may be the NH<sub>2</sub>-terminal domain. The polypeptide linker may be of variable length and composition so long as the two variable domains are bridged without serious steric interference. Typically, the linkers are comprised primarily of stretches of glycine and serine residues with some glutamic acid or lysine residues interspersed for solubility. ScFvs are encompassed within the term “antigen-binding fragment.”

Diabodies are dimeric scFvs. The components of diabodies typically have shorter peptide linkers than most scFvs, and they show a preference for associating as dimers (*see, e.g.*, Holliger, P., et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R. J., et al. (1994) Structure 2: 1121-1123). Diabodies are also encompassed within the term “antigen-binding fragment.”

A Fv fragment is an antigen binding fragment which consists of one VH and one VL domain held together by noncovalent interactions. Although the two domains of the Fv fragment, VL and VH, can be coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); *see, e.g.*, Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc.

Natl. Acad. Sci. USA 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding fragment" of an antibody. The term dsFv is used herein to refer to an Fv with an engineered intermolecular disulfide bond to stabilize the VH-VL pair. dsFvs are also encompassed within the term "antigen-binding fragment."

5        A F(ab')<sub>2</sub> fragment is an antigen binding fragment essentially equivalent to that obtained from immunoglobulins (typically IgG) by digestion with an enzyme pepsin at pH 4.0-4.5. The fragment may be recombinantly produced. F(ab')<sub>2</sub> are also encompassed within the term "antigen-binding fragment."

10        A Fab fragment is an antigen binding fragment essentially equivalent to that obtained by reduction of the disulfide bridge or bridges joining the two heavy chain pieces in the F(ab')<sub>2</sub> fragment. The Fab' fragment may be recombinantly produced. Fab' are also encompassed within the term "antigen-binding fragment."

15        A Fab fragment is an antigen binding fragment essentially equivalent to that obtained by digestion of immunoglobulins (typically IgG) with the enzyme papain. The Fab fragment may be recombinantly produced. The heavy chain segment of the Fab fragment is the Fd piece. Fab fragments are also encompassed within the term "antigen-binding fragment."

20        An affibody is a small protein comprising a three-helix bundle that functions as an antigen binding molecule (*e.g.*, an antibody mimetic). Generally, affibodies are approximately 58 amino acids in length and have a molar mass of approximately 6 kDa. Affibody molecules with unique binding properties are acquired by randomization of 13 amino acids located in two alpha-helices involved in the binding activity of the parent protein domain. Specific affibody molecules binding a desired target protein can be isolated from pools (libraries) containing billions of different variants, using methods such as phage display. Affibodies are also encompassed within the term "antigen-binding fragment."

25        The term "human antibody" refers to antibodies having variable and constant regions corresponding substantially to, or derived from, antibodies obtained from human subjects, *e.g.*, encoded by human germline immunoglobulin sequences or variants thereof. Human antibodies may include one or more amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis in vitro or by  
30        somatic mutation in vivo). Such mutations may present in one or more of the CDRs, particularly CDR3, or in one or more of the framework regions. In some embodiments, the human antibodies may have at least one, two, three, four, five, or more positions replaced with an amino acid residue that is not encoded by the human germline immunoglobulin sequence. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR

sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library (Hoogenboom H. R., (1997) TIB Tech. 15:62-70; Azzazy H., and Highsmith W. E., (2002) Clin. Biochem. 35:425-445; Gavilondo J. V., and Larrick J. W. (2002) BioTechniques 29: 128-145; Hoogenboom H., and Chames P. (2000) Immunology Today 21:371-378), antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (*see, e.g.*, Taylor, L. D., et al. (1992) Nucl. Acids Res. 20:6287-6295; Kellermann S-A., and Green L. L. (2002) Current Opinion in Biotechnology 13:593-597; Little M. et al (2000) Immunology Today 21:364-370) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions as defined above. In certain embodiments, however, such recombinant human antibodies may be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies may be sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain comprising an amino acid sequence represented by any one of SEQ ID NOs: 8, 10, 27, and 29. In some embodiments, the anti-RAN antibodies and antigen binding fragments of the disclosure comprise a light chain comprising an amino acid sequence represented by any one of SEQ ID NOs: 13, 15, 32, and 34.

In some embodiments, the anti-RAN antibodies or antigen binding fragments may or may not include the framework region of the antibodies, for example the framework region amino acid sequences. In some embodiments, anti-RAN antibodies are murine antibodies. In some embodiments, anti-RAN antibodies are chimeric or humanized antibodies.

In some embodiments, the antibody or antigen binding fragment comprises a VH sequence as set forth in any one of SEQ ID NOs: 7, 17, and 26. In some embodiments, the antibody or antigen binding fragment comprises a VL sequence as set forth in any one of SEQ ID NOs: 12, 18, or 31.

In some embodiments, the antibody or antigen binding fragment comprises a VH sequence as set forth in SEQ ID NO. 7 and a VL sequence as set forth in SEQ ID NO. 12.

In some embodiments, the antibody or antigen binding fragment comprises a VH sequence as set forth in SEQ ID NO. 17 and a VL sequence as set forth in SEQ ID NO. 18.

In some embodiments, the antibody or antigen binding fragment comprises a VH sequence as set forth in SEQ ID NO. 26 and a VL sequence as set forth in SEQ ID NO. 31.

5 In some embodiments, antibody or antigen-binding fragment comprises six complementarity determining regions (CDRs): CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, wherein CDRH1 comprises a sequence as set forth in SEQ ID NO: 1, CDRH2 comprises a sequence as set forth in SEQ ID NO: 2, CDRH3 comprises a sequence as set forth in SEQ ID NO: 3, CDRL1 comprises a sequence as set forth in SEQ ID NO: 4, CDRL2  
10 comprises a sequence as set forth in SEQ ID NO: 5, and CDRL3 comprises a sequence as set forth in SEQ ID NO: 6.

In some embodiments, antibody or antigen-binding fragment comprises six complementarity determining regions (CDRs): CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, wherein CDRH1 comprises a sequence as set forth in SEQ ID NO: 20, CDRH2  
15 comprises a sequence as set forth in SEQ ID NO: 21, CDRH3 comprises a sequence as set forth in SEQ ID NO: 22, CDRL1 comprises a sequence as set forth in SEQ ID NO: 23, CDRL2 comprises a sequence as set forth in SEQ ID NO: 24, and CDRL3 comprises a sequence as set forth in SEQ ID NO: 25.

It should be appreciated that, in some embodiments, the disclosure contemplates variants  
20 (*e.g.*, homologs) of amino acid and nucleic acid sequences for the heavy chain variable region and light chain variable region of the antibodies. "Homology" refers to the percent identity between two polynucleotides or two polypeptide moieties. The term "substantial homology", when referring to a nucleic acid, or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary  
25 strand), there is nucleotide sequence identity in about 90 to 100% of the aligned sequences. For example, in some embodiments, nucleic acid sequences sharing substantial homology are at least 90%, at least 91%, at least 92% at least 93%, at least 94%, at least 95%, at least 96% at least 97%, at least 98% at least 99% sequence identity. When referring to a polypeptide, or fragment thereof, the term "substantial homology" indicates that, when optimally aligned with  
30 appropriate gaps, insertions or deletions with another polypeptide, there is nucleotide sequence identity in about 90 to 100% of the aligned sequences. The term "highly conserved" means at least 80% identity, preferably at least 90% identity, and more preferably, over 97% identity. For example, in some embodiments, highly conserved proteins share at least 85%, at least 90%, at least 91%, at least 92% at least 93%, at least 94%, at least 95%, at least 96% at least 97%, at  
35 least 98% at least 99% identity. In some cases, highly conserved may refer to 100% identity.

Identity is readily determined by one of skill in the art by, for example, the use of algorithms and computer programs known by those of skill in the art.

In some embodiments, RAN antibodies of the disclosure can bind to a RAN protein with high affinity, *e.g.*, with a  $K_d$  less than  $10^{-7}$  M,  $10^{-8}$  M,  $10^{-9}$  M,  $10^{-10}$  M,  $10^{-11}$  M or lower. For example, anti-RAN antibodies or antigen binding fragments can bind to a RAN protein with an affinity between 5 pM and 500 nM, *e.g.*, between 50 pM and 100 nM, *e.g.*, between 500 pM and 50 nM. The disclosure also includes antibodies or antigen binding fragments that compete with any of the antibodies described herein for binding to RAN proteins and that have an affinity of 50 nM or lower (*e.g.*, 20 nM or lower, 10 nM or lower, 500 pM or lower, 50 pM or lower, or 5 pM or lower). The affinity and binding kinetics of the anti-RAN protein antibody can be tested using any method known in the art including but not limited to biosensor technology (*e.g.*, OCTET or BIACORE).

In some embodiments, anti-RAN antibodies of the present disclosure include the VH, VL, and CDR, amino acid sequences shown in Table 2 below.

Table 2: Representative sequences of developed anti-poly(GA) monoclonal antibodies

|              | Antibody   |            |            |           |           |
|--------------|------------|------------|------------|-----------|-----------|
|              | 27B11 IgG1 | 27B11 IgG2 | 27B11 ScFv | 23H2 IgG1 | 23H2 IgG2 |
| HC CDR1      | 1          | 1          | 1          | 20        | 20        |
| HC CDR2      | 2          | 2          | 2          | 21        | 21        |
| HC CDR3      | 3          | 3          | 3          | 22        | 22        |
| LC CDR1      | 4          | 4          | 4          | 23        | 23        |
| LC CDR2      | 5          | 5          | 5          | 24        | 24        |
| LC CDR3      | 6          | 6          | 6          | 25        | 25        |
| HC Variable* | 7          | 7          | 17         | 26        | 26        |
| LC Variable* | 12         | 12         | 18         | 31        | 31        |
| Heavy Chain* | 8          | 10         | N/A        | 27        | 29        |
| Light Chain* | 13         | 15         | N/A        | 32        | 34        |

In some embodiments, antibody clone 27B11 binds to poly(GA). In some embodiments, clone 27B11 is an IgG1 antibody. In some embodiments, clone 27B11 is an IgG2 antibody. In some embodiments, clone 27B11 is an scFv. In some embodiments, antibody clone 23H2 binds to poly(GA). In some embodiments, antibody clone 23H2 is an IgG1 antibody. In some embodiments, antibody clone 23H2 is an IgG2 antibody.

Anti-RAN antibodies may be used to treat, or assist in the treatment of, one or more symptoms of a disease associated with RAN proteins. In some embodiments, the disease associated with RAN proteins is selected from the group consisting of: amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), and frontotemporal dementia. In some  
5     embodiments, the neurological disease associated with RAN proteins is Alzheimer's Disease (AD).

In some embodiments, the anti-RAN antibodies may be used to treat, or assist in the treatment of, one or more symptoms of a disease associated with RAN proteins, for example by administering a therapeutically effective amount of one or more anti-RAN antibodies to a  
10     subject diagnosed as having one or more symptoms of a disease associated with RAN proteins (*e.g.*, the early stages of Alzheimer's disease) or being at risk of developing a disease associated with RAN proteins (*e.g.*, based on one or more assays described in this application).

To "treat" a disease (*e.g.*, a disease associated with poly(GA) RAN translation, for example Alzheimer's disease) as the term is used herein, means to reduce the frequency or  
15     severity of at least one sign or symptom of a disease or disorder experienced by a subject. The compositions described above or elsewhere herein are typically administered to a subject in an effective amount, that is, an amount capable of producing a desirable result. The desirable result will depend upon the active agent being administered. A therapeutically acceptable amount of an anti-RAN protein antibody may be an amount that is capable of treating a disease, *e.g.*,  
20     Alzheimer's disease, by reducing expression and/or aggregation of RAN proteins and/or appearance or number of RNA foci comprising RAN protein-encoding microsatellite repeat sequences. As is well known in the medical and veterinary arts, dosage for any one subject depends on many factors, including the subject's size, body surface area, age, the particular composition to be administered, the active ingredient(s) in the composition, time and route of  
25     administration, general health, and other drugs being administered concurrently.

In some embodiments, one or more of the anti-RAN antibody or antigen binding fragments disclosed herein are administered to a subject, wherein the subject has been characterized as having a disease associated with RAN proteins by the detection of at least one RAN protein in a biological sample obtained from the subject.

#### *Anti-RAN Antibody Production*

Typically, polyclonal antibodies are produced by inoculation of a suitable mammal, such as a mouse, rabbit or goat. An antigen is injected into the mammal. This induces the B-lymphocytes to produce IgG immunoglobulins specific for the antigen. This polyclonal IgG is  
35     purified from the mammal's serum. Monoclonal antibodies are generally produced by a single

cell line (*e.g.*, a hybridoma cell line). In some embodiments, an anti-RAN antibody is purified (*e.g.*, isolated from serum). In some embodiments, an antigen comprises a poly(GA) RAN protein repeat sequence.

Numerous methods may be used for obtaining anti-RAN antibodies. For example, antibodies can be produced using recombinant DNA methods. Monoclonal antibodies may also be produced by generation of hybridomas (*see, e.g.*, Kohler and Milstein (1975) *Nature*, 256: 495-499) in accordance with known methods. Hybridomas formed in this manner are then screened using standard methods, such as enzyme-linked immunosorbent assay (ELISA; *e.g.*, RCA-based ELISA or rtPCR-based ELISA) and surface plasmon resonance (*e.g.*, OCTET or BIACORE) analysis, to identify one or more hybridomas that produce an antibody that specifically binds with a specified antigen. Any form of the specified antigen (*e.g.*, a RAN protein) may be used as the immunogen, *e.g.*, recombinant antigen, naturally occurring forms, any variants or fragments thereof. One exemplary method of making antibodies includes screening protein expression libraries that express antibodies or fragments thereof (*e.g.*, scFv), *e.g.*, phage or ribosome display libraries. Phage display is described, for example, in Ladner et al., U.S. Pat. No. 5,223,409; Smith (1985) *Science* 228: 1315-1317; Clackson et al. (1991) *Nature*, 352: 624-628; Marks et al. (1991) *J. Mol. Biol.*, 222: 581-597; WO 91/17271; WO 92/20791; WO 92/15679; WO 93/01288; WO 92/01047; WO 92/09690; and WO 90/02809.

In another embodiment, a monoclonal antibody is obtained from the non-human animal, and then modified, *e.g.*, made chimeric, using recombinant DNA techniques known in the art. A variety of approaches for making chimeric antibodies have been described. *See, e.g.*, Morrison et al., *Proc. Natl. Acad. Sci. U.S.A.* 81:6851, 1985; Takeda et al., *Nature* 314:452, 1985; Cabilly et al., U.S. Pat. No. 4,816,567; Boss et al., U.S. Pat. No. 4,816,397; Tanaguchi et al., European Patent Publication EP171496; European Patent Publication 0173494, United Kingdom Patent GB 2177096B.

Antibodies can also be humanized by methods known in the art. For example, monoclonal antibodies with a desired binding specificity can be commercially humanized (Scotgene, Scotland; and Oxford Molecular, Palo Alto, Calif.). Fully humanized antibodies, such as those expressed in transgenic animals are within the scope of the invention (*see, e.g.*, Green et al. (1994) *Nature Genetics* 7, 13; and U.S. Patent Nos. 5,545,806 and 5,569,825). For additional antibody production techniques, *see*, *Antibodies: A Laboratory Manual*, Second Edition. Edited by Edward A. Greenfield, Dana-Farber Cancer Institute, ©2014. The present disclosure is not necessarily limited to any particular source, method of production, or other special characteristics of an antibody.

Some aspects of the present disclosure relate to isolated cells (*e.g.*, host cells) transformed with a polynucleotide or vector. In some embodiments, the vector is an rAAV vector. In some embodiments, the host cells are transduced or infected with an rAAV particle comprising an rAAV vector encoding an anti-poly(GA) antibody.

5           Host cells may be a prokaryotic or eukaryotic cell. The polynucleotide or vector which is present in the host cell may either be integrated into the genome of the host cell or it may be maintained extrachromosomally. The host cell can be any prokaryotic or eukaryotic cell, such as a bacterial, insect, fungal, plant, animal or human cell. In some embodiments, fungal cells are, for example, those of the genus *Saccharomyces*, in particular those of the species *S. cerevisiae*.  
10       The term "prokaryotic" includes all bacteria which can be transformed or transfected with a DNA or RNA molecules for the expression of an antibody or the corresponding immunoglobulin chains. Prokaryotic hosts may include gram negative as well as gram positive bacteria such as, for example, *E. coli*, *S. typhimurium*, *Serratia marcescens* and *Bacillus subtilis*. The term "eukaryotic" includes yeast, higher plants, insects and vertebrate cells, *e.g.*, mammalian cells,  
15       such as NSO and CHO cells. Depending upon the host employed in a recombinant production procedure, the antibodies or immunoglobulin chains encoded by the polynucleotide may be glycosylated or may be non-glycosylated. Antibodies or the corresponding immunoglobulin chains may also include an initial methionine amino acid residue.

          In some embodiments, once a vector has been incorporated into an appropriate host, the  
20       host may be maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the immunoglobulin light chains, heavy chains, light/heavy chain dimers or intact antibodies, antigen binding fragments or other immunoglobulin forms may follow; *see*, Beychok, Cells of Immunoglobulin Synthesis, Academic Press, N.Y., (1979). Thus, polynucleotides or vectors are introduced into the cells  
25       which in turn produce the antibody or antigen binding fragments. Furthermore, transgenic animals, preferably mammals, comprising the aforementioned host cells may be used for the large scale production of the antibody or antibody fragments.

          The transformed host cells can be grown in fermenters and cultured according to techniques known in the art to achieve optimal cell growth. Once expressed, the whole  
30       antibodies, their dimers, individual light and heavy chains, other immunoglobulin forms, or antigen binding fragments, can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like; *see*, Scopes, "Protein Purification", Springer Verlag, N.Y. (1982). The antibody or antigen binding fragments can then be isolated from the growth medium, cellular lysates, or  
35       cellular membrane fractions. The isolation and purification of the, *e.g.*, microbially expressed

antibodies or antigen binding fragments may be by any conventional means such as, for example, preparative chromatographic separations and immunological separations such as those involving the use of monoclonal or polyclonal antibodies directed, *e.g.*, against the constant region of the antibody.

5 Aspects of the disclosure relate to a hybridoma, which provides an indefinitely prolonged source of monoclonal antibodies. As used herein, "hybridoma cell" refers to an immortalized cell derived from the fusion of B lymphoblasts with a myeloma fusion partner. For preparing monoclonal antibody-producing cells (*e.g.*, hybridoma cells), an individual animal whose antibody titer has been confirmed (*e.g.*, a mouse) is selected, and 2 days to 5 days after the final  
10 immunization, its spleen or lymph node is harvested and antibody-producing cells contained therein are fused with myeloma cells to prepare the desired monoclonal antibody producer hybridoma. Measurement of the antibody titer in antiserum can be carried out, for example, by reacting the labeled protein, as described hereinafter and antiserum and then measuring the activity of the labeling agent bound to the antibody. The cell fusion can be carried out according  
15 to known methods, for example, the method described by Kochler and Milstein (Nature 256:495 (1975)). As a fusion promoter, for example, polyethylene glycol (PEG) or Sendai virus (HVJ) is used.

Examples of myeloma cells include NS-1, P3U1, SP2/0, AP-1 and the like. The proportion of the number of antibody producer cells (spleen cells) and the number of myeloma  
20 cells to be used is preferably about 1:1 to about 20:1. PEG (preferably PEG 1000-PEG 6000) is preferably added in concentration of about 10% to about 80%. Cell fusion can be carried out efficiently by incubating a mixture of both cells at about 20°C to about 40 °C, preferably about 30 °C to about 37 °C for about 1 minute to 10 minutes.

Various methods may be used for screening for a hybridoma producing the antibody  
25 (*e.g.*, against a tumor antigen or autoantibody of the present invention). For example, where a supernatant of the hybridoma is added to a solid phase (*e.g.*, microplate) to which antibody is adsorbed directly or together with a carrier and then an anti-immunoglobulin antibody (if mouse cells are used in cell fusion, anti-mouse immunoglobulin antibody is used) or Protein A labeled with a radioactive substance or an enzyme is added to detect the monoclonal antibody against  
30 the protein bound to the solid phase. Alternately, a supernatant of the hybridoma is added to a solid phase to which an anti-immunoglobulin antibody or Protein A is adsorbed and then the protein labeled with a radioactive substance or an enzyme is added to detect the monoclonal antibody against the protein bound to the solid phase.

Selection of the monoclonal antibody can be carried out according to any known method  
35 or its modification. Normally, a medium for animal cells to which HAT (hypoxanthine,

aminopterin, thymidine) are added is employed. Any selection and growth medium can be employed as long as the hybridoma can grow. For example, RPMI 1640 medium containing 1% to 20%, preferably 10% to 20% fetal bovine serum, GIT medium containing 1% to 10% fetal bovine serum, a serum free medium for cultivation of a hybridoma (SFM-101, Nissui Seiyaku) and the like can be used. Normally, the cultivation is carried out at 20°C to 40°C, preferably 37°C for about 5 days to 3 weeks, preferably 1 week to 2 weeks under about 5% CO<sub>2</sub> gas. The antibody titer of the supernatant of a hybridoma culture can be measured according to the same manner as described above with respect to the antibody titer of the anti-protein in the antiserum.

As an alternative to obtaining immunoglobulins directly from the culture of hybridomas, immortalized hybridoma cells can be used as a source of rearranged heavy chain and light chain loci for subsequent expression and/or genetic manipulation. Rearranged antibody genes can be reverse transcribed from appropriate mRNAs to produce cDNA. If desired, the heavy chain constant region can be exchanged for that of a different isotype or eliminated altogether. The variable regions can be linked to encode single chain Fv regions. Multiple Fv regions can be linked to confer binding ability to more than one target or chimeric heavy and light chain combinations can be employed. Any appropriate method may be used for cloning of antibody variable regions and generation of recombinant antibodies.

In some embodiments, an appropriate nucleic acid that encodes variable regions of a heavy and/or light chain is obtained and inserted into an expression vectors which can be transfected into standard recombinant host cells. A variety of such host cells may be used. In some embodiments, mammalian host cells may be advantageous for efficient processing and production. Typical mammalian cell lines useful for this purpose include CHO cells, 293 cells, or NSO cells. The production of the antibody or antigen binding fragment may be undertaken by culturing a modified recombinant host under culture conditions appropriate for the growth of the host cells and the expression of the coding sequences. The antibodies or antigen binding fragments may be recovered by isolating them from the culture. The expression systems may be designed to include signal peptides so that the resulting antibodies are secreted into the medium; however, intracellular production is also possible.

The disclosure also includes a polynucleotide encoding at least a variable region of an immunoglobulin chain of the antibodies described herein. In some embodiments, the variable region encoded by the polynucleotide comprises at least one complementarity determining region (CDR) of the VH and/or VL of the variable region of the antibody produced by any one of the above described hybridomas.

Polynucleotides encoding antibody or antigen binding fragments may be, *e.g.*, DNA, cDNA, RNA or synthetically produced DNA or RNA or a recombinantly produced chimeric

nucleic acid molecule comprising any of those polynucleotides either alone or in combination. In some embodiments, a polynucleotide is part of a vector. Such vectors may comprise further genes such as marker genes which allow for the selection of the vector in a suitable host cell and under suitable conditions.

5           In some embodiments, a polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells. Expression of the polynucleotide comprises transcription of the polynucleotide into a translatable mRNA. Regulatory elements ensuring expression in eukaryotic cells, preferably mammalian cells, are well known to those skilled in the art. They may include regulatory sequences that facilitate  
10   initiation of transcription and optionally poly-A signals that facilitate termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers, and/or naturally associated or heterologous promoter regions. Possible regulatory elements permitting expression in prokaryotic host cells include, *e.g.*, the PL, Lac, Trp or Tac promoter in *E. coli*, and examples of regulatory elements  
15   permitting expression in eukaryotic host cells are the AOX1 or GAL1 promoter in yeast or the CMV-promoter, SV40-promoter, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells.

          Beside elements which are responsible for the initiation of transcription such regulatory elements may also include transcription termination signals, such as the SV40-poly-A site or the  
20   tk-poly-A site, downstream of the polynucleotide. Furthermore, depending on the expression system employed, leader sequences capable of directing the polypeptide to a cellular compartment or secreting it into the medium may be added to the coding sequence of the polynucleotide and are well known in the art. The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader  
25   sequence capable of directing secretion of translated protein, or a portion thereof, into, for example, the extracellular medium. Optionally, a heterologous polynucleotide sequence can be used that encode a fusion protein including a C- or N-terminal identification peptide imparting desired characteristics, *e.g.*, stabilization or simplified purification of expressed recombinant product.

30           In some embodiments, polynucleotides encoding at least the variable domain of the light and/or heavy chain may encode the variable domains of both immunoglobulin chains or only one. Likewise, polynucleotides may be under the control of the same promoter or may be separately controlled for expression. Furthermore, some aspects relate to vectors, particularly plasmids, cosmids, viruses and bacteriophages used conventionally in genetic engineering that  
35   comprise a polynucleotide encoding a variable domain of an immunoglobulin chain of an

antibody or antigen binding fragment; optionally in combination with a polynucleotide that encodes the variable domain of the other immunoglobulin chain of the antibody.

In some embodiments, expression control sequences are provided as eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of the polynucleotides or vector into targeted cell population (*e.g.*, to engineer a cell to express an antibody or antigen binding fragment). A variety of appropriate methods can be used to construct recombinant viral vectors. In some embodiments, polynucleotides and vectors can be reconstituted into liposomes for delivery to target cells. The vectors containing the polynucleotides (*e.g.*, the heavy and/or light variable domain(s) of the immunoglobulin chains encoding sequences and expression control sequences) can be transferred into the host cell by suitable methods, which vary depending on the type of cellular host.

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#### *Pharmaceutical Compositions*

In some aspects, the disclosure relates to pharmaceutical compositions comprising rAAV vectors or rAAVs (*e.g.*, rAAVs encoding anti-RAN antibodies or antigen binding fragments). In some embodiments, the composition comprises an rAAV vector or rAAV particle, and a pharmaceutically acceptable carrier. As used herein the term “pharmaceutically acceptable carrier” is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions. Pharmaceutical compositions can be prepared as described below. The active ingredients may be admixed or compounded with any conventional, pharmaceutically acceptable carrier or excipient. The compositions may be sterile.

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Typically, pharmaceutical compositions are formulated for delivering an effective amount of an agent (*e.g.*, an rAAV encoding an anti-RAN antibody). In general, an “effective amount” of an active agent refers to an amount sufficient to elicit the desired biological response (*e.g.*, ameliorating one or more symptoms of ALS). An effective amount of an agent may vary depending on such factors as the desired biological endpoint, the pharmacokinetics of the

compound, the disease being treated (*e.g.*, ALS, repeat expansion diseases), the mode of administration, and the patient.

A composition is said to be a “pharmaceutically acceptable carrier” if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known in the art. *See*, for example, REMINGTON'S PHARMACEUTICAL SCIENCES, 18th Ed. (1990).

It will be understood by those skilled in the art that any mode of administration, vehicle or carrier conventionally employed and which is inert with respect to the active agent may be utilized for preparing and administering the pharmaceutical compositions of the present disclosure. Illustrative of such methods, vehicles and carriers are those described, for example, in Remington's Pharmaceutical Sciences, 4th ed. (1970), the disclosure of which is incorporated herein by reference. Those skilled in the art, having been exposed to the principles of the disclosure, will experience no difficulty in determining suitable and appropriate vehicles, excipients and carriers or in compounding the active ingredients therewith to form the pharmaceutical compositions of the disclosure.

An effective amount, also referred to as a therapeutically effective amount, of a compound (*e.g.*, an rAAV encoding an anti-RAN antibody) is an amount sufficient to ameliorate at least one adverse effect associated with a disease associated with RAN proteins, such as, *e.g.*, memory loss, cognitive impairment, loss of coordination, speech impairment, *etc.* In some embodiments, the neurological disease associated with RAN proteins is selected from the group consisting of: amyotrophic lateral sclerosis (ALS), frontotemporal dementia, and Alzheimer's disease. In a specific embodiment, the neurological disease associated with RAN proteins is ALS. The therapeutically effective amount to be included in pharmaceutical compositions depends, in each case, upon several factors, *e.g.*, the type, size and condition of the patient to be treated, the intended mode of administration, the capacity of the patient to incorporate the intended dosage form, *etc.* Generally, an amount of active agent is included in each dosage form to provide from about 0.1 to about 250 mg/kg, and preferably from about 0.1 to about 100 mg/kg. One of ordinary skill in the art would be able to determine empirically an appropriate therapeutically effective amount. In some embodiments, the effective amount of rAAV included in a pharmaceutical composition is at least  $1 \times 10^5$  vector genomes (vg), such as wherein the pharmaceutical composition is administered to a subject and the effective amount of the rAAV in the pharmaceutical composition is  $1 \times 10^5$  vg per kilogram (kg) (vg/kg) of the subject's body weight. For example, in some embodiments, rAAV is administered to a subject at an amount of about  $1 \times 10^6$  vg/kg to  $10^{10}$  vg/kg or more (*e.g.*,  $2 \times 10^6$  vg/kg,  $3 \times 10^6$  vg/kg,  $4 \times 10^6$  vg/kg,  $5 \times 10^6$  vg/kg,  $6 \times 10^6$  vg/kg,  $7 \times 10^6$  vg/kg,  $8 \times 10^6$  vg/kg,  $9 \times 10^6$  vg/kg,  $1 \times 10^7$  vg/kg,  $2 \times 10^7$

vg/kg,  $3 \times 10^7$  vg/kg,  $4 \times 10^7$  vg/kg,  $5 \times 10^7$  vg/kg,  $6 \times 10^7$  vg/kg,  $7 \times 10^7$  vg/kg,  $8 \times 10^7$  vg/kg,  $9 \times 10^7$  vg/kg,  $1 \times 10^8$  vg/kg,  $2 \times 10^8$  vg/kg,  $3 \times 10^8$  vg/kg,  $4 \times 10^8$  vg/kg,  $5 \times 10^8$  vg/kg,  $6 \times 10^8$  vg/kg,  $7 \times 10^8$  vg/kg,  $8 \times 10^8$  vg/kg,  $9 \times 10^8$ ,  $1 \times 10^9$  vg/kg,  $2 \times 10^9$  vg/kg,  $3 \times 10^9$  vg/kg,  $4 \times 10^9$  vg/kg,  $5 \times 10^9$  vg/kg,  $6 \times 10^9$  vg/kg,  $7 \times 10^9$  vg/kg,  $8 \times 10^9$  vg/kg,  $9 \times 10^9$  vg/kg, vg/kg,  $1 \times 10^{10}$  vg/kg,  $2 \times 10^{10}$  vg/kg,  $3 \times 10^{10}$  vg/kg,  $4 \times 10^{10}$  vg/kg,  $5 \times 10^{10}$  vg/kg,  $6 \times 10^{10}$  vg/kg,  $7 \times 10^{10}$  vg/kg,  $8 \times 10^{10}$  vg/kg,  $9 \times 10^{10}$  vg/kg, or more). In some embodiments, rAAV is administered to a subject at an amount of about  $1 \times 10^6$  vg/kg to  $1 \times 10^{14}$  vg/kg,  $1 \times 10^8$  vg/kg to  $1 \times 10^{13}$  vg/kg, or  $1 \times 10^9$  vg/kg to  $1 \times 10^{12}$  vg/kg.

Combined with the teachings provided herein, by choosing among the various active compounds and weighing factors such as potency, relative bioavailability, patient body weight, severity of adverse side-effects and selected mode of administration, an effective prophylactic or therapeutic treatment regimen can be planned which does not cause substantial toxicity and yet is entirely effective to treat the particular subject. The effective amount for any particular application can vary depending on such factors as the disease or condition being treated, the particular therapeutic agent being administered, the size of the subject, or the severity of the disease or condition. One of ordinary skill in the art can empirically determine the effective amount of a particular nucleic acid and/or other therapeutic agent without necessitating undue experimentation.

The term pharmaceutically-acceptable carrier means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration to a human or other vertebrate animal. The term carrier denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being commingled with the compounds of the present disclosure, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficiency.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Suitable liquid or solid pharmaceutical preparation forms are, for example, aqueous or saline solutions for inhalation, microencapsulated, encochleated, coated onto microscopic gold particles, contained in liposomes, nebulized, aerosols, pellets for implantation into the skin, or dried onto a sharp object to be scratched into the skin. The pharmaceutical compositions also include granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, drops or preparations with protracted release of active

compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of methods for drug delivery, *see*, Langer R (1990) *Science* 249:1527-1533, which is incorporated herein by reference.

The compounds may be administered *per se* (neat) or in the form of a pharmaceutically acceptable salt. When used in medicine the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof. Such salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulphonic, tartaric, citric, methane sulphonic, formic, malonic, succinic, naphthalene-2-sulphonic, and benzene sulphonic. Also, such salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the compounds into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the compounds into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product. Liquid dose units are vials or ampoules. Solid dose units are tablets, capsules and suppositories.

#### *Administration and Medical Uses*

A therapeutic agent may be delivered by any suitable modality known in the art.

Aspects of the disclosure relate to the delivery of a therapeutically effective amount of a therapeutic agent to a subject. In some embodiments, a therapeutically effective amount is an amount effective in reducing repeat expansions in the subject. In some embodiments, a therapeutically effective amount is an amount effective in reducing the transcription of RNAs that produce RAN proteins in a subject. In certain embodiments, a therapeutically effective amount is an amount effective in reducing the translation of RAN proteins in a subject. In some embodiments, a therapeutically effective amount is an amount effective for treating a disease associated with repeat expansions. "Reducing" expression of a repeat sequence or RAN protein translation refers to a decrease in the amount or level of repeat sequence expression or RAN

protein translation in a subject after administration of a therapeutic agent (and relative to the amount or level in the subject prior to the administration).

Accordingly, aspects of the disclosure further relate to rAAV vectors, rAAVs, and pharmaceutical compositions for use in administration to a subject (e.g., for use in treating a  
5 subject having or suspected of having a disease associated with RAN protein translation or RAN protein accumulation) and use of rAAV vectors and rAAVs in the manufacture of a medicament (e.g., a medicament for treating a subject having or suspected of having a disease associated with RAN protein translation or RAN protein accumulation).

In certain embodiments, the effective amount is an amount effective in reducing the level  
10 of RAN proteins by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% (e.g., the level of RAN proteins relative to the level of RAN proteins in a cell or subject that has not been administered a therapeutic agent). In certain embodiments, the effective amount is an amount effective in reducing the translation of RAN proteins by at least 10%, at least 20%, at least 30%,  
15 at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% (e.g., the level of RAN proteins relative the level of RAN proteins in a cell or subject that has not been administered a therapeutic agent).

Pharmaceutical compositions described herein can be prepared by any method known in the art of pharmacology. In general, such preparatory methods include bringing the compound  
20 described herein (*i.e.*, the “active ingredient”) into association with a carrier or excipient, and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping, and/or packaging the product into a desired single- or multi-dose unit.

Pharmaceutical compositions can be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. A “unit dose” is a discrete amount of the  
25 pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage, such as one-half or one-third of such a dosage.

Relative amounts of the active ingredient, the pharmaceutically acceptable excipient,  
30 and/or any additional ingredients in a pharmaceutical composition described herein will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. The composition may comprise between 0.1% and 100% (w/w) active ingredient.

Therapeutic agents described herein are typically formulated in dosage unit form for ease  
35 of administration and uniformity of dosage. It will be understood, however, that the total daily

usage of the compositions described herein will be decided by a physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject or organism will depend upon a variety of factors including the disease being treated and the severity of the disorder; the activity of the specific active ingredient employed; the specific composition employed; the age, body weight, general health, sex, and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific active ingredient employed; the duration of the treatment; drugs used in combination or coincidental with the specific active ingredient employed; and like factors well known in the medical arts.

A therapeutic agent can be administered by any route, including enteral (*e.g.*, oral), parenteral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, subcutaneous, intraventricular, intracerebroventricular, transdermal, intradermal, rectal, intravaginal, intraperitoneal, topical (as by powders, ointments, creams, and/or drops), mucosal, nasal, buccal, sublingual; by intratracheal instillation, bronchial instillation, and/or inhalation; and/or as an oral spray, nasal spray, and/or aerosol. Specifically contemplated routes are oral administration, intravenous administration (*e.g.*, systemic intravenous injection), regional administration *via* blood and/or lymph supply, and/or direct administration to an affected site. In general, the most appropriate route of administration will depend upon a variety of factors including the nature of the agent (*e.g.*, its stability in the environment of the gastrointestinal tract), and/or the condition of the subject (*e.g.*, whether the subject is able to tolerate oral administration).

In some embodiments, administration of an rAAV described herein to a subject having or suspected of having a RAN protein-associated disease (*e.g.*, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), or frontotemporal dementia) reduces the expression and/or aggregation of RAN proteins (*e.g.*, poly(GA) RAN proteins) in one or more central nervous system tissues (*e.g.*, the cortex, the frontal cortex, the motor cortex, retrosplenial cortex, and/or the hippocampus). In some embodiments, administration of an rAAV described herein reduces the expression and/or aggregation of RAN proteins (*e.g.*, poly(GA) RAN proteins) in the cortex, the frontal cortex, the motor cortex, retrosplenial cortex, and/or the hippocampus of a subject. In some embodiments, administration of an rAAV described herein increases motor neuron survival in a subject (*e.g.*, a subject characterized as having or suspected of having a RAN protein-associated disease, such as Alzheimer's disease, amyotrophic lateral sclerosis (ALS), or frontotemporal dementia). In some embodiments, administration of an rAAV described herein increases Fc receptor levels in a subject, such as Fc receptor levels in one or more central nervous system tissues (*e.g.*, the cortex, the frontal cortex, the motor cortex, retrosplenial cortex, and/or the hippocampus). In some embodiments, administration of an rAAV described herein to a subject increases Fc receptor levels when the subject expresses a RAN protein, such as a

poly(GA) RAN protein (e.g., a subject characterized as having or suspected of having a RAN protein-associated disease, such as Alzheimer's disease, amyotrophic lateral sclerosis (ALS), or frontotemporal dementia).

The exact amount of a therapeutic agent required to achieve an effective amount will vary from subject to subject, depending, for example, on species, age, and general condition of a subject, severity of the side effects or disorder, identity of the particular compound, mode of administration, and the like. An effective amount may be included in a single dose (e.g., single oral dose) or multiple doses (e.g., multiple oral doses). In certain embodiments, when multiple doses are administered to a subject or applied to a biological sample, tissue, or cell, any two doses of the multiple doses include different or substantially the same amounts of a compound described herein. In certain embodiments, when multiple doses are administered to a subject or applied to a biological sample, tissue, or cell, the frequency of administering the multiple doses to the subject or applying the multiple doses to the biological sample, tissue, or cell is three doses a day, two doses a day, one dose a day, one dose every other day, one dose every third day, one dose every week, one dose every two weeks, one dose every three weeks, or one dose every four weeks. In certain embodiments, the frequency of administering the multiple doses to the subject or applying the multiple doses to the biological sample, tissue, or cell is one dose per day. In certain embodiments, the frequency of administering the multiple doses to the subject or applying the multiple doses to the biological sample, tissue, or cell is two doses per day. In certain embodiments, the frequency of administering the multiple doses to the subject or applying the multiple doses to the biological sample, tissue, or cell is three doses per day. In certain embodiments, when multiple doses are administered to a subject or applied to a biological sample, tissue, or cell, the duration between the first dose and last dose of the multiple doses is one day, two days, four days, one week, two weeks, three weeks, one month, two months, three months, four months, six months, eight months, nine months, one year, two years, three years, four years, five years, seven years, ten years, fifteen years, twenty years, or the lifetime of the subject, tissue, or cell. In certain embodiments, the duration between the first dose and last dose of the multiple doses is three months, six months, or one year. In certain embodiments, the duration between the first dose and last dose of the multiple doses is the lifetime of the subject, tissue, or cell.

In some embodiments, a treatment for a disease associated with RAN protein expression is administered to the central nervous system (CNS) of a subject in need thereof. As used herein, the "central nervous system (CNS)" refers to all cells and tissues of the brain and spinal cord of a subject, including but not limited to neuronal cells, glial cells, astrocytes, cerebrospinal fluid, *etc.* Modalities of administering a therapeutic agent to the CNS of a subject include direct

injection into the brain (*e.g.*, intracerebral injection, intraventricular injection, intraparenchymal injection, *etc.*), direct injection into the spinal cord of a subject (*e.g.*, intrathecal injection, lumbar injection, *etc.*), or any combination thereof.

In some embodiments, a treatment as described by the disclosure is systemically administered to a subject, for example by intravenous injection. Systemically administered therapeutic molecules can be modified, in some embodiments, in order to improve delivery of the molecules to the CNS of a subject. Examples of modifications that improve CNS delivery of therapeutic molecules include but are not limited to co-administration or conjugation to blood brain barrier-targeting agents (*e.g.*, transferrin, melanotransferrin, low-density lipoprotein (LDL), angiopeps, RVG peptide, *etc.*, as disclosed by Georgieva et al. Pharmaceuticals 6(4): 557-583 (2014)), coadministration with BBB disrupting agents (*e.g.*, bradykinins), and physical disruption of the BBB prior to administration (*e.g.*, by MRI-Guided Focused Ultrasound), *etc.*

The following Examples are intended to illustrate the benefits of the present invention and to describe particular embodiments but are not intended to exemplify the full scope of the invention. Accordingly, it will be understood that the Examples are not meant to limit the scope of the invention.

## EXAMPLES

This Example describes expression and testing of AAV-based monoclonal antibodies that bind to poly(GA) RAN proteins in a C9orf72 mutant mouse model of amyotrophic lateral sclerosis (C9 BAC). C9orf72 mouse models (C9 BAC) of ALS received either a control treatment (PBS) or a recombinant AAV9 virus comprising a transgene encoding a recombinant  $\alpha$ -poly(GA) antibody (IgG1 A, ScFv, IgG1 B, or IgG2) at 30-37 weeks, 37-44 weeks, or 44-50 weeks of age prior to immunohistochemistry analyses. FIG. 1 shows representative immunohistochemistry (IHC) data indicating that anti-poly(GA)-IgG1 antibodies expressed from rAAVs reduce the number of poly(GA) RAN protein aggregates in a C9 BAC of ALS. These observations were further confirmed by quantifications of GA aggregates which were analyzed by one-way ANOVA with Sidak analyses for multiple comparisons. FIG. 2 shows representative data indicating that anti-poly(GA)-IgG1 antibodies expressed from rAAVs reduce the number of poly(GA) RAN protein aggregates in a C9orf72 mouse model of ALS. Further analyses were performed to confirm that anti-poly(GA)-IgG1 antibodies target poly(GA) RAN protein aggregates. FIG. 3 shows representative microscopy data indicating that AAV-based anti-poly(GA) antibodies co-localize with poly(GA) RAN protein aggregates in the C9 BAC mouse model.

FIG. 4 shows representative data obtained from meso-scale discovery (MSD) immunoassays using monoclonal mouse  $\alpha$ -GP antibody (capture) and rabbit polyclonal  $\alpha$ -GP antibody (detection) in frontal cortex from treated animals. These results indicated AAV9-based delivery of anti-poly(GA) antibodies reduces total number of GP levels in the C9 BAC mouse model by mechanisms which can involve improving protein homeostasis.

FIGs. 5A-5B show representative data indicating AAV9-based delivery of anti-poly(GA) antibodies reduces neuroinflammation in the motor cortex of C9 BAC mouse brains. FIG. 5A shows immunohistochemistry showing GFAP staining as a marker of neuroinflammation in brain tissues obtained from PBS-treated NT mice and C9 animals treated with PBS or antibodies. FIG. 5B shows quantifications of the data shown in FIG. 5A.

FIG. 6 shows representative data indicating novel  $\alpha$ -GA antibody recognize GA aggregates in C9-BAC mouse brain and human patient-derived cells (iMN). HEK293 cells were engineered to comprise a nucleic acid encoding an  $\alpha$ -GA antibody. The media was changed at 24 hours post-transfection and protein lysates and supernatant were obtained at 48 hours post-transfection. These samples were subsequently used in western blot and fluorescence microscopy analyses to verify the expression levels of ScFv-FLAG, IgG1-A, IgG1-B and IgG2. FIGs. 7A-7B show representative data indicating  $\alpha$ -GA recombinant antibodies are expressed and secreted. FIG. 7A shows western blot analyses of cell protein lysates collected from HEK293 cells. FIG. 7B shows fluorescence microscopy analyses of supernatant comprising primary  $\alpha$ -GA antibodies collected from collected from HEK293 cells.

FIG. 8 shows representative data indicating recombinant antibodies recognize GA aggregates in C9-mouse brain.

HEK293 cells were engineered to comprise a nucleic acid encoding an  $\alpha$ -GA antibody and a nucleic acid encoding GFP-GA<sub>60</sub>. The media was changed at 24 hours post-transfection and protein lysates and supernatant were obtained at 48 hours post-transfection. These samples were subsequently used in western blot and fluorescence microscopy analyses. FIGs. 9A-9B show representative data indicating recombinant  $\alpha$ -GA antibodies reduce GFP-GA<sub>60</sub> in HEK293T cells. FIG. 9A shows western blot analyses of protein lysates obtained from cells expressing  $\alpha$ -GA recombinant antibodies and GFP-GA<sub>60</sub>. FIG. 9B shows a quantification of the data shown in FIG. 9A. FIGs. 10A-10B shows representative data indicating recombinant  $\alpha$ -GA antibodies reduce GA aggregate number and size. FIG. 10A shows fluorescence microscopy analyses of protein lysates obtained from cells expressing  $\alpha$ -GA recombinant antibodies and GFP-GA<sub>60</sub>. FIG. 10B shows quantification of the data shown in FIG. 10B.

FIG. 11A shows a diagram of a recombinant  $\alpha$ -GA immunoglobulin and a nucleic acid comprising a transgene encoding the recombinant  $\alpha$ -GA immunoglobulin. FIG. 11B shows a

diagram of  $\alpha$ -GA single chain variable fragment (scFV) and a nucleic acid comprising a transgene encoding the  $\alpha$ -GA scFV. A recombinant AAV-antibody against green fluorescent protein was developed as an Isotype control for in vivo experiments which will allow assessment of effects of non-GA targeting vs. GA-targeting antibody treatments. FIGs. 11C-11D show the development of a recombinant AAV AAV1 ERDR-IgG-GFP isotype control. FIG. 11C shows a diagram of the  $\alpha$ -GFP isotype control and a nucleic acid comprising a transgene encoding the  $\alpha$ -GFP isotype control. FIG. 11D shows representative data indicating from fluorescence microscopy analyses of the  $\alpha$ -GFP isotype control. The  $\alpha$ -GFP isotype control was delivered by an rAAV comprising a VP1 capsid protein of serotype AAV1 and comprising an ERDRTRG (SEQ ID NO: 49) peptide insertion. The VP1 protein was encoded by the nucleic acid of SEQ ID NO: 43 and comprised the amino acid sequence of SEQ ID NO: 44.

FIG. 12 shows a non-limiting example of an efficacy study strategy using AAV1-ERDR-IgG C9-BAC mice.

FIG. 13 shows representative data indicating wide-spread delivery of AAV1-ERDR-TFP after ICV injection.

FIGs. 14A-14B show representative data obtained from hanging wire tests of injected mouse subjects. FIG. 14A shows hanging wire scores from analyses of injected mouse subjects. FIG. 14B shows photographs taken during hanging wire analyses of injected mouse subjects. Further results from hanging wire performance analyses are shown in FIGs. 24A-24B, which indicate that AAV-GA antibody improves hanging wire performance (FIG. 24A) and reduces GA levels in the brain of C9-BAC male mice (FIG. 24B).

FIGs. 15A-15F show representative data obtained from open-field analyses of injected mouse subjects. FIG. 15A shows open field analysis of C9orf72 positive mice showing variable ambulatory phenotypes. FIG. 15B shows representative data from open field analyses of treated and untreated C9orf72 BAC mice (ambulatory parameters). FIG. 15C shows representative data from open field analyses of treated and untreated C9orf72 BAC mice (stereotypic and exploratory behavior). FIG. 15D shows representative data indicating treatment B rescues 11/12 open field abnormalities in C9-BAC mice compared to vehicle treatment. FIG. 15E shows representative data indicating Group B treated C9-BAC mice show no differences in open field behavior compared to NT animals. FIG. 15F shows representative data indicating treatment A modestly improves 4 stereotypic grooming/exploration but not ambulation parameters in C9-BAC mice.

FIG. 16 shows representative data indicating treatment B improves survival of C9-BAC mice compared to treatment A and treatment with vehicle.

FIG. 17 shows representative data indicating treatment A reduces GA aggregates.

Brain and spinal cord tissues were harvested from mouse subjects that received no treatment or were administered either vehicle, an AAV comprising a nucleic acid encoding a recombinant control antibody, or an AAV comprising a nucleic acid encoding a recombinant  $\alpha$ -GA IgG1. Lysates generated from the tissue samples were diluted and subjected to assays for measuring the levels of  $\alpha$ -GA IgG1. FIG. 18 shows representative data obtained from analyses of the distribution of recombinant  $\alpha$ -GA antibody which indicate AAV-based delivery of  $\alpha$ -GA antibody to mouse subjects results in the presence of the antibody in the brain and spinal cord tissues.

FIGs. 19A-19B show representative data indicating AAV-based delivery of recombinant  $\alpha$ -GA antibody reduces GA aggregates in the frontal cortex of C9-BAC mice. In addition, FIGs. 20A-20B show representative data indicating AAV-based delivery of recombinant  $\alpha$ -GA antibody increases motor neuron survival in C9-BAC mouse brains.

Further microscopy analyses were performed which confirmed the delivery of  $\alpha$ -GA antibody to the brains of treated mouse subjects. FIGs. 21A-21B show representative data obtained from microscopy analyses of C9-BAC mouse brains indicating recombinant  $\alpha$ -GA antibody is retained in the CA2 region after AAV-based delivery. FIGs. 22A-22B show representative data obtained from microscopy analyses of C9-BAC mouse brains indicating AAV-based delivery of recombinant  $\alpha$ -GA antibody in mouse brains.

In addition, FIGs. 23A-23B show AAV-GA antibody treatment reduces GA levels in C9 female mice brain (FIG. 23A) and Organoids (FIG. 23B) derived from C9orf72 ALS/FTD.

#### REPRESENTATIVE SEQUENCES

> Anti-polyGA antibody clone 27B11 heavy chain CDR1 amino acid sequence (SEQ ID NO: 1)  
GFAFSNYG

> Anti-polyGA antibody clone 27B11 heavy chain CDR2 amino acid sequence (SEQ ID NO: 2)  
INSDGDST

> Anti-polyGA antibody clone 27B11 heavy chain CDR3 amino acid sequence (SEQ ID NO: 3)  
ARVGGNYDFAMDY

> Anti-polyGA antibody clone 27B11 light chain CDR1 amino acid sequence (SEQ ID NO: 4)  
QSLLNSRTRKNY

> Anti-polyGA antibody clone 27B11 light chain CDR2 amino acid sequence (SEQ ID NO: 5)  
WTS

> Anti-polyGA antibody clone 27B11 light chain CDR3 amino acid sequence (SEQ ID NO: 6)

KQSYNNPWT

> Anti-polyGA antibody clone 27B11 heavy chain variable region amino acid sequence (SEQ ID NO: 7)

5 EVQLQESGGGSVQPGGSLKLSCAASGFAFSNYGMSWVRQTPDKRLELVTITNSDGDSTF  
YPDSVKGRFTISRDNALYQMSSSLKSDDTAMYYCARVGGNYDFAMDYWGQGT  
SIVSS

> Anti-polyGA antibody clone 27B11 heavy chain IgG1 amino acid sequence (SEQ ID NO: 8)

10 EVQLQESGGGSVQPGGSLKLSCAASGFAFSNYGMSWVRQTPDKRLELVTITNSDGDSTF  
YPDSVKGRFTISRDNALYQMSSSLKSDDTAMYYCARVGGNYDFAMDYWGQGT  
SIVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFP  
AVLQSDLYTLSSSVTPSSTWPSETVTCNVAHPASSTKVDKIVPRDCGCKPCICTVPEV  
SSVFIFPPKPKDVLITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFN  
15 STFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQ  
MAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQK  
SNWEAGNTFTCSVLHEGLHNHHTKSLSHSPGK

> Anti-polyGA antibody clone 27B11 heavy chain IgG1 amino acid sequence with signal peptide (SEQ ID NO: 9)

20 MDWTWRVFCLLAVAPGAHSEVQLQESGGGSVQPGGSLKLSCAASGFAFSNYGMSWV  
RQTPDKRLELVTITNSDGDSTFYYPDSVKGRFTISRDNALYQMSSSLKSDDTAMYYC  
ARVGGNYDFAMDYWGQGTIVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYF  
PEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTPSSTWPSETVTCNVAHPASSTKV  
25 DKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLITLTPKVTCVVVDISKDDPEVQFSW  
FVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTIS  
KTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQ  
PIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVLHEGLHNHHTKSLSHSPGK

30 > Anti-polyGA antibody clone 27B11 heavy chain IgG2 amino acid sequence (SEQ ID NO: 10)

EVQLQESGGGSVQPGGSLKLSCAASGFAFSNYGMSWVRQTPDKRLELVTITNSDGDSTF  
YPDSVKGRFTISRDNALYQMSSSLKSDDTAMYYCARVGGNYDFAMDYWGQGT  
SIVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPA  
VLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPN  
35 LLGGPSVFIFPPKIKDVLMISSLPIVTCVVVDVSEDDPDVQISWVNNVEVHTAQTQTHR  
EDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLP  
PPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTPEVLDSGSYFMYSK  
LRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

40 > Anti-polyGA antibody clone 27B11 heavy chain IgG2 amino acid sequence with signal peptide (SEQ ID NO: 11)

MKCSWVIFFLMAVVIGINSEVQLQESGGGSVQPGGSLKLSCAASGFAFSNYGMSWVRQ  
TPDKRLELVTITNSDGDSTFYYPDSVKGRFTISRDNALYQMSSSLKSDDTAMYYCAR  
VGGNYDFAMDYWGQGTIVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEP  
45 VTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKK

IEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS  
WVFNNEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIE  
RTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNY  
KNTEPVLDSGYSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

5

> Anti-polyGA antibody clone 27B11 light chain variable region amino acid sequence (SEQ ID NO: 12)

DIVMSQFPSSLAVSAGDKVTMSCKSSQSLLNSRTRKKNYLAWYQQKPGQSPKLLIYWTS  
TRESGVPDRFTGSRSGTDFTLTISVQAEDLAVYYCKQSYNNPWTFGGGKLEIK

10

> Anti-polyGA antibody clone 27B11 light chain IgG1 amino acid sequence (SEQ ID NO: 13)

DIVMSQFPSSLAVSAGDKVTMSCKSSQSLLNSRTRKKNYLAWYQQKPGQSPKLLIYWTS  
TRESGVPDRFTGSRSGTDFTLTISVQAEDLAVYYCKQSYNNPWTFGGGKLEIKRADA  
APTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSRQNGVLNSWTDQDSKD  
STYSMSSTLTLTKEYERHNSYTCEATHKTSTSPIVKSFNRECEC

15

> Anti-polyGA antibody clone 27B11 light chain IgG1 amino acid sequence with signal peptide (SEQ ID NO: 14)

MKLPVRLLVLMFWIPASSSDIVMSQFPSSLAVSAGDKVTMSCKSSQSLLNSRTRKKNYL  
WYQQKPGQSPKLLIYWTSTRESGVPDRFTGSRSGTDFTLTISVQAEDLAVYYCKQSYN  
NPWTFGGGKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGS  
ERQNGVLNSWTDQDSKDESTYSMSSTLTLTKEYERHNSYTCEATHKTSTSPIVKSFNRECEC

20

> Anti-polyGA antibody clone 27B11 light chain IgG2 amino acid sequence (SEQ ID NO: 15)

DIVMSQFPSSLAVSAGDKVTMSCKSSQSLLNSRTRKKNYLAWYQQKPGQSPKLLIYWTS  
TRESGVPDRFTGSRSGTDFTLTISVQAEDLAVYYCKQSYNNPWTFGGGKLEIKRADA  
APTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSRQNGVLNSWTDQDSKD  
STYSMSSTLTLTKEYERHNSYTCEATHKTSTSPIVKSFNRECEC

25

> Anti-polyGA antibody clone 27B11 light chain IgG2 amino acid sequence with signal peptide (SEQ ID NO: 16)

MKLPVRLLVLMFWIPASSSDIVMSQFPSSLAVSAGDKVTMSCKSSQSLLNSRTRKKNYL  
WYQQKPGQSPKLLIYWTSTRESGVPDRFTGSRSGTDFTLTISVQAEDLAVYYCKQSYN  
NPWTFGGGKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGS  
ERQNGVLNSWTDQDSKDESTYSMSSTLTLTKEYERHNSYTCEATHKTSTSPIVKSFNRECEC

30

> Anti-polyGA antibody clone 27B11 scFv heavy chain (SEQ ID NO: 17)

EVQLQESGGGSGVQPGGSLKLSCAASGFAFSNYGMSWVRQTPDKRLELVTINSDGDSSTF  
YPDSVKGRFTISRDNALYQLQMSSSLKSDDTAMYYCARVGGNYDFAMDYWGQGTSTVIVSS

40

> Anti-polyGA antibody clone 27B11 scFv light chain (SEQ ID NO: 18)

DIVMSQFPSSLAVSAGDKVTMSCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWTS  
TRESGVPDRFTGSRSGTDFLTITSSVQAEDLAVYYCKQSYNNPWTFGGGTKLEIK

- 5 > Anti-polyGA antibody clone 27B11 scFv complete sequence with signal peptide and poly-GS linker (SEQ ID NO: 19)

MDWTWRVFCLLAVAPGAHSEVQLQESGGGSGVQPGGSLKLSCAASGFAFSNYGMSWV  
RQTPDKRLELVTTINSDGDSTFYPD SVKGRFTISRDN AKNALYLMSSLKSDDTAMY YC  
ARVGGNYDFAMDYWGQGT SVIVSSGGGGSGGGGSGGGGSDIVMSQFPSSLAVSAGDK  
VTMSCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWTS  
10 TLTISVQAEDLAVYYCKQSYNNPWTFGGGTKLEIK

> Anti-polyGA antibody clone 23H2 heavy chain CDR1 amino acid sequence (SEQ ID NO: 20)  
GFTFSSHG

- 15 > Anti-polyGA antibody clone 23H2 heavy chain CDR2 amino acid sequence (SEQ ID NO: 21)  
INSNGGST

> Anti-polyGA antibody clone 23H2 heavy chain CDR3 amino acid sequence (SEQ ID NO: 22)  
ARVGDNDDFAMGY

- 20 > Anti-polyGA antibody clone 23H2 light chain CDR1 amino acid sequence (SEQ ID NO: 23)  
QSLFNSRTRKNY

> Anti-polyGA antibody clone 23H2 light chain CDR2 amino acid sequence (SEQ ID NO: 24)  
25 WTS

> Anti-polyGA antibody clone 23H2 light chain CDR3 amino acid sequence (SEQ ID NO: 25)  
KQSYNNPWT

- 30 > Anti-polyGA antibody clone 23H2 heavy chain variable region amino acid sequence (SEQ ID NO: 26)  
EVQLQESGGGSGVQPGGALQLSCAASGFTFSSHGMSWVRQTPDKRLEMVATINSNGGST  
YYPDSVKGRFIISRDN AKNTLYLMSSLKSEDTAMY YCARVGDNDDFAMGYWGQGT  
VTVSS

35 > Anti-polyGA antibody clone 23H2 heavy chain IgG1 amino acid sequence (SEQ ID NO: 27)  
EVQLQESGGGSGVQPGGALQLSCAASGFTFSSHGMSWVRQTPDKRLEMVATINSNGGST  
YYPDSVKGRFIISRDN AKNTLYLMSSLKSEDTAMY YCARVGDNDDFAMGYWGQGT  
VTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFP  
40 AVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEV  
SSVFIFPPKPKDVLITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFN  
STFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQ  
MAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQK  
SNWEAGNTFTCSVLHEGLHNHTEKSLSHSPGK

45

> Anti-polyGA antibody clone 23H2 heavy chain IgG1 amino acid sequence with signal peptide (SEQ ID NO: 28)

MDWTWRVFCLLAVAPGAHSEVQLQESGGGSVQPGGALQLSCAASGFTFSSHGMSWVR  
QTPDKRLEMVATINSNGGSTYYPD SVKGRFIISRDNAKNTLYLQMSSLKSEDTAMYCY  
5 ARVGDNDDFAMGYWGQGT SVTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYF  
PEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKV  
DKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLITLTPKVTCVVVDISKDDPEVQFSW  
FVDDDEVHTAQTQPREEQFNSTFRSVSELPIHQDWLNGKEFKCRVNSAAFPAPIEKTIS  
KTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQ  
10 PIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVLHEGLHNHHTKSLSHSPGK

> Anti-polyGA antibody clone 23H2 heavy chain IgG2 amino acid sequence (SEQ ID NO: 29)

EVQLQESGGGSVQPGGALQLSCAASGFTFSSHGMSWVRQTPDKRLEMVATINSNGGST  
YYPD SVKGRFIISRDNAKNTLYLQMSSLKSEDTAMYCYCARVGDNDDFAMGYWGQGT  
15 VTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTF  
AVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAP  
NLLGGPSVFIFPPKIKDVLMLISLPIVTCVVVDVSEDDPDVQISWVFNNEVHTAQTQTH  
REDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVL  
PPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGKTELNYKNTEPVLDSGYSYFMYSK  
20 LRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

> Anti-polyGA antibody clone 23H2 heavy chain IgG2 amino acid sequence with signal peptide (SEQ ID NO: 30)

MDWTWRVFCLLAVAPGAHSEVQLQESGGGSVQPGGALQLSCAASGFTFSSHGMSWVR  
25 QTPDKRLEMVATINSNGGSTYYPD SVKGRFIISRDNAKNTLYLQMSSLKSEDTAMYCY  
ARVGDNDDFAMGYWGQGT SVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYF  
PEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKV  
DKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMLISLPIVTCVVVDVSEDDPD  
VQISWVFNNEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL  
30 APIERTISKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGKTE  
LNYKNTEPVLDSGYSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPG  
K

> Anti-polyGA antibody clone 23H2 light chain variable region amino acid sequence (SEQ ID NO: 31)

35 DIVMSQSPSSLAVSEGEKVTLTCKSSQSLFNSRTRKKNYLAWYQQKPGQPPKLLIYWTST  
RESGVPDRFTGSGYGTDFTLTISVQAEDLAVYYCKQSYNNPWTFGGGKLEIK

> Anti-polyGA antibody clone 23H2 light chain IgG1 amino acid sequence (SEQ ID NO: 32)

40 DIVMSQSPSSLAVSEGEKVTLTCKSSQSLFNSRTRKKNYLAWYQQKPGQPPKLLIYWTST  
RESGVPDRFTGSGYGTDFTLTISVQAEDLAVYYCKQSYNNPWTFGGGKLEIKRADA  
APTVISIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSRQNGVLNSWTDQDSK  
STYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

> Anti-polyGA antibody clone 23H2 light chain IgG1 amino acid sequence with signal peptide (SEQ ID NO: 33)

MKLPVRLLVLMFWIPASSSDIVMSQSPSSLAVSEGEKVTLTCKSSQSLFNSRTRKNYLA  
WYQQKPGQPPKLLIYWTSTRESGVPDRFTGSGYGTDFTLTISVQAEDLAVYYCKQSYN  
5 NPWTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNFPKDINVKWKIDGS  
ERQNGVLNSWTDQDSKDYMSSTLTTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRN  
EC

> Anti-polyGA antibody clone 23H2 light chain IgG2 amino acid sequence (SEQ ID NO: 34)

10 DIVMSQSPSSLAVSEGEKVTLTCKSSQSLFNSRTRKNYLAWYQQKPGQPPKLLIYWTST  
RESGVPDRFTGSGYGTDFTLTISVQAEDLAVYYCKQSYNNPWTFGGGTKLEIKRADA  
APTVSIFPPSSEQLTSGGASVVCFLNFPKDINVKWKIDGSERQNGVLNSWTDQDSK  
DYMSSTLTTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

15 > Anti-polyGA antibody clone 23H2 light chain IgG2 amino acid sequence with signal peptide (SEQ ID NO: 35)

MKLPVRLLVLMFWIPASSSDIVMSQSPSSLAVSEGEKVTLTCKSSQSLFNSRTRKNYLA  
WYQQKPGQPPKLLIYWTSTRESGVPDRFTGSGYGTDFTLTISVQAEDLAVYYCKQSYN  
NPWTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNFPKDINVKWKIDGS  
20 ERQNGVLNSWTDQDSKDYMSSTLTTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRN  
EC

> Anti-polyGA antibody clone 27B11 IgG1 nucleic acid sequence (with signal peptide and bGH poly(A) signal) (SEQ ID NO: 36)

25 atggattggacttgagagtggtttgcctgctggctgtcgcacctggagctcatagtgaggtgcagctgcaggagctcgggggaggtcag  
tgcagcctggagggtccctgaaactctctgcgcagcctctggattcgtttcagtaactatggcatgcttgggttcgccagactccagaca  
agaggctggagttggtcacaaccattaatagtgatggtgatagtagacctttatccagacagtgtaagggccgattccactctccagagaca  
atgccaagaacgccctgtacctgcaaatgagcagctgaagtcagacgacacagccatgtattactgtcgaagagtgaggagtaactacg  
actttgctatggactactgggtcagggaaacctcagtcagtcgtcctcaGCTAAACGACACCCCCATCTGTCTA  
30 TCCACTGGCCCCTGGATCTGCTGCCCAAACCTAACTCgATGGTGACCCTGGGATGCCT  
GGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACCTCTGGTTCCCTGTC  
CAGCGGTGTGCACACCTTCCCAGCTGTCCTcCAGTCTGACCTCTACACTCTGAGCAG  
CTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGC  
CCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTT  
35 GTAAGCCTTGCATATGcACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCAA  
AGCCCAAGGATGTGCTCACCATTACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAG  
ACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGTTTGTAGATGATGTGGAG  
GTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTC  
AGTCAGTGAACCTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGTTCAAAT  
40 GCAGGGTCAACAGTGCAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAACC  
AAAGGCAGACCGAAGGCTCCGCAGGTcTACACCATTCACCTCCCAAGGAGCAGAT  
GGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTCCCTGAAGACA  
TACTGTGGAGTGGCAGTGGAAATGGGCAGCCAGCGGAGAACTACAAGAACTCA  
GCCCATCATGGACACAGATGGCTCTTACTTCGTCTACAGCAAGCTCAATGTGCAGA  
45 AGAGCAACTGGGAGGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTG

CACAACCACCATACTGAGAAGAGCCTCTCCCCTCTCCTGGTAAAgtgaaacagactttgaattt  
tgaccttctcaagttggcgggaGACGTCgagtccaacctggaccATGAAGTTGCCTGTTAGGCTGTTGGT  
GCTGATGTTCTGGATCCCTGCTTCCAGCAGTGACATTGTGATGTCACAGTTTCCATC  
CTCCCTGGCTGTGTCAGCAGGAGATAAGGTCATATGAGCTGCAAATCCAGTCAGA  
5 GTCTGCTCAACAGTAGGACCCGAAAGAACTACTTGGCTTGGTAiCAGCAGAAACCA  
GGGCAGTCTCCTAAACTACTGATCTACTGGACATCCACTCGGGAATCTGGcGTCCCT  
GATCGCTTCACAGGCAGTCGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTa  
CAGGCTGAAGACCTGGCAGTTTATTACTGCAAGCAATCTTATAATAATCCGTGGAC  
GTTCTGGTGGAGGCACCAAGCTiGAAATAAAACGGGCAGATGCTGCAcCAACTGTATC  
10 gATCTTCCCACCATCCAGTGAGCAGTTAACATCcGGAGGTGCCTCAGTCGTGTGCTTC  
TTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGA  
ACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCT  
ACAGCATGAGCAGCACCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGC  
TATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAaAGCTTCAAC  
15 AGGAATGAGTGTTAGgGCGCCCACTGTGCTGGATATCGTTTAAACCGCTGATCAG  
CCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTC  
CTTGACCCTGGAAGGTGCCACTCCCCTGTCCTTTCTTAATAAAATGAGGAAATTGC  
ATCGCATTGTCTGAGTAGGTGTCTATTCTGGGGGGTGGGGTGGGGCAGGACA  
GCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTC  
20 TATGG

> Anti-polyGA antibody clone 27B11 IgG2 nucleic acid sequence (with signal peptide and bGH  
poly(A) signal) (SEQ ID NO: 37)

ATGAAATGCAGCTGGGTCATCTTCTTCCTGATGGCAGTGTTATAGGAATCAATTCA  
25 gaggtgcagctgcaggagtctgggggaggctcagtcagcctggagggtccctgaaactctctgcgcagcctctggattcgcttcagt  
aactatggcatgtcttgggttcgccagactccagacaagaggtggagttggtcacaaccattaatagtgatggtgatagtagtacccttatccag  
acagtgtaggggccgattcaccatctccagagacaatgccagaacgcccgtacctgcaaatgagcagctctgaagtcagacgacaca  
gccatgtattactgtgcaagagtgaggagtaactacgactttgctatggactactggggtcagggaacctcagtcacgtgtcctcaGCC  
AAAACAACAGCCCCATCGGTCTATCCACTGGCCCCTGTGTGTGGAGATACAACCTGG  
30 CTCCTCGGTGACcCTAGGATGCCTGGTCAAGGGTTATTTCCCTGAGCCAGTGACCTT  
GACCTGGAACCTCTGGATCgCTGTCCAGTGGTGTGCACACCTTCCCAGCTGTCCTGCA  
GTCTGACCTCTACACCCTgAGCAGCTCAGTGAACCTCcAGCACCTGGCCCAG  
CCAGTCCATCACCTGCAATGTGGCCCAACCGGCAAGCAGCACCAAGGTGGACAAGA  
AAATTGAGCCCAGAGGGCCCAATCAAGCCCTGTCCTCCATGCAAATGCCAGCA  
35 CCTAACCTCTTGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGATGTA  
CTCATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGGAT  
GACCCAGATGTCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCA  
GACACAAACCCATAGAGAGGATTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCC  
CCATCCAGCACCAGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAAC  
40 AAAGACCTCCCAGCGCCCATCGAGAGAACCATCTCAAAACCCAAAGGGTCAGTAAG  
AGCTCCACAGGTATATGTCTTGCCTCCACCAGAAGAAGAGATGACTAAGAAACAGG  
TCACTCTGACCTGCATGGTCACAGACTTCATGCCTGAAGACATTTACGTGGAGTGGA  
CCAACAACGGGAAAACAGAGCTAAACTACAAGAACTGAACCAGTCCTGGACTCT  
GATGGTTCTTACTTCATGTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGA  
45 AAGAAATAGCTACTCCTGTTCACTGGTCCACGAGGGTCTGCACAATCACACACGA

CTAAGAGCTTCTCCCGGACTCCGGGTAAAgtgaaacagactttgaattttgaccttctcaagttggcgggagacg  
tggagtccaacctggacctATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATcCCT  
GCTTCCAGCAGTgacattgtgatgtcacagttccatcctccctggctgtgtcagcaggagataaggtcactatgagctgcaaatc  
cagtcagagtctgctcaacagtaggacccgaaagaactacttggcttggtaccagcagaaccagggcagttcctaaactactgatctact  
5 ggacatccactcgggaatctggcgtccctgatcgttcacaggcagtcgatctgggacagatttcactctcaccatcagcagtggtgcaggct  
gaagacctggcagtttattactgcaagcaatcttataataatccgtggacgttcgggtggaggcaccaagcttgaaataaaaCGGGCTG  
ATGCTGCACCAACTGTATCgATCTTCCCACCATCCAGTGAGCAGTTAACATCcGGAG  
GTGCCCTCAGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGT  
GGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAG  
10 GACAGCAAAGACAGCACCTACAGCATGAGCAGCACCTCACGTTGACCAAGGACG  
AGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCA  
CCCATTGTCAAaAGCTTCAACAGGAATGAGTGTTAGgcGGCCGCCACTGTGCTGGAT  
ATCGTTTAAACCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTG  
TTTGCCCTCCCCCGTGCCTTCCCTTGACCCTGGAAGGTGCCACTCCCAGTGTCCCTTC  
15 CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCAATTCTATTCTGGG  
GGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCAT  
GCTGGGGATGCGGTGGGCTCTATGG

> Anti-polyGA antibody clone 27B11 ScFv nucleic acid sequence (with signal peptide, FLAG,  
20 and bGH poly(A) signal) (SEQ ID NO: 38)  
ATGGATTGGACTTGGAGAGTGTTTTGCTGCTGGCTGTCTGCACCTGGGGCTCATAGT  
GAGGTGCAGCTGCAGGAGTCTGGGGGAGGCTCAGTGCAGCCTGGAGGGTCCCTGAA  
ACTCTCCTGCGCAGCCTCTGGATTTCGCTTTCAGTAACTATGGCATGTCTTGGGTTCG  
CCAGACTCCAGACAAGAGGCTGGAGTTGGTCACAACCATTAAATAGTGATGGTGATA  
25 GTACCTTTTATCCAGACAGTGTGAAGGGCCGATTACCATCTCCAGAGACAATGCC  
AAGAACGCCCTGTACCTGCAAATGAGCAGTCTGAAGTCAGACGACACAGCCATGTA  
TACTGTGCAAGAGTGGGAGGTAACCTACGACTTTGCTATGGACTACTGGGGTCAGG  
GAACCTCAGTCATCGTCTCCTCAGGTGGAGGCGGTTACAGGCGGAGGTGGCAGCGGC  
GGTGGCGGGTTCGGACATTGTGATGTACAGTTTCCATCCTCCCTGGCTGTGTACAGCA  
30 GGAGATAAGGTCATATGAGCTGCAAATCCAGTCAGAGTCTGCTCAACAGTAGGAC  
CCGAAAGAACTACTTGGCTTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTAC  
TGATCTACTGGACATCCACTCGGGAATCTGGGGTCCCTGATCGCTTCACAGGCAGTC  
GATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCA  
GTTTATTACTGCAAGCAATCTTATAATAATCCGTGGACGTTTCGGTGGAGGCACCAAG  
35 CTGGAAATAAAAgattataaagatcatgatggcgattataaagatcatgatattgattataaagatgatgatataaataagcGG  
CCGCCACTGTGCTGGATATCGTTTAAACCGCTGATCAGCCTCGACTGTGCCTTCTAG  
TTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCCCTTGACCCTGGAAGGTGCC  
ACTCCCAGTGCCTTTCCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGG  
TGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGA  
40 AGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG

> Anti-polyGA antibody clone 23H2 IgG1 nucleic acid sequence (with signal peptide and bGH  
poly(A) signal) (SEQ ID NO: 39)  
atggattggacttgagagtggtttgcctgctggctgtcgcacctggagctcatagtgaggtgcagctgcaggagtctgggggaggtcag  
45 tgcagcctggagggccctgcaactctcctgtgcagcctctggattcatttcagtagtcatggcatgtcttgggttcgccagactccagaca

agaggctggaaatggtcgcaaccattaatagtaatggtgggagtacattaccagacagtgatgaaggccgattcatcatccagaga  
caatgccaaaaacacctgtacctgcaaatgagcagctctgaagtctgaggacacagccatgtattactgtgcaagagtgggagataacgac  
gactttgctatgggctactggggtcaaggaacctcagtcaccgtgtcctcaGCTAAAACGACACCCCCATCTGTCT  
ATCCACTGGCCCCCTGGATCTGCTGCCCAAACCTAACTCgATGGTGACCCTGGGATGCC  
5 TGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACCTCTGGTTCCCTGT  
CCAGCGGTGTGCACACCTTCCCAGCTGTCCTcCAGTCTGACCTCTACACTCTGAGCA  
GCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTG  
CCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGT  
TGTAAGCCTTGCATATGcACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCAA  
10 AGCCCAAGGATGTGCTCACCATTACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAG  
ACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGTTTGTAGATGATGTGGAG  
GTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTC  
AGTCAGTGAACCTTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGTTCAAAT  
GCAGGGTCAACAGTGCAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAACC  
15 AAAGGCAGACCGAAGGCTCCGCAGGTcTACACCATTCACCTCCCAAGGAGCAGAT  
GGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTCCCTGAAGACA  
TTACTGTGGAGTGGCAGTGAATGGGCAGCCAGCGGAGAACTACAAGAACTCA  
GCCCATCATGGACACAGATGGCTCTTACTTCGTCTACAGCAAGCTCAATGTGCAGA  
AGAGCAACTGGGAGGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTG  
20 CACAACCACCATACTGAGAAGAGCCTCTCCCACTCTCCTGGTAAAgtgaaacagactttgaattt  
tgaccttctcaagttggcgaggGACGTCgagtcacacctggacccATGAAGTTGCCTGTTAGGCTGTTGGT  
GCTGATGTTCTGGATCCCTGCTTCCAGCAGTgacattgtgatgtcacagtcctcctcctggtgtgcaga  
aggagagaaggtcactttaacctgcaaatccagtcagagttttcaacagtagaacccgaaagaactacttggttggtaccagcagaaa  
ccagggcagcctcctaaactgttgatctactggacatccactagggaatctggggtccctgatcgcttcacaggcagtggtatgggacag  
25 atttactctcaccatcagcagtggtgcaggtgaagacctggcagtttattactgcaacaactcttataataatccgtggacgttcggtggagg  
caccaagcttgaataaaaCGGGCAGATGCTGCAcCAACTGTATCgATCTTCCCACCATCCAGT  
GAGCAGTTAACATCcGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTTCTACCCC  
AAAGACATCAATGTCAAGTGAAGATTGATGGCAGTGAACGACAAAAATGGCGTCTCT  
GAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACC  
30 CTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCAC  
TCACAAGACATCAACTTCACCCATTGTCAAaAGCTTCAACAGGAATGAGTGTTAGGc  
GGCCGCCACTGTGCTGGATATCGTTTAAACCGCTGATCAGCCTCGACTGTGCCTTCT  
AGTTGCCAGCCATCTGTTGTTTGCCCCCCCCCGTGCCCTTCTTGACCCTGGAAGGT  
GCCACTCCCCTGTCTTTTCTTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGT  
35 AGGTGTCAATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTG  
GGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG

> Anti-polyGA antibody clone 23H2 IgG2 nucleic acid sequence (with signal peptide and bGH  
poly(A) signal) (SEQ ID NO: 40)

40 atggattggacttgagagtggtttgcctgctggctgtcgacactggagctcatagtgaggtgcagctgcaggagtctgggggaggctcag  
tgcagcctggaggggcccctgcaactctctgtgcagcctctggattcacttcagtagtcatggcatgtcttgggttcgccagactccagaca  
agaggctggaaatggtcgcaaccattaatagtaatggtgggagtacattaccagacagtgatgaaggccgattcatcatccagaga  
caatgccaaaaacacctgtacctgcaaatgagcagctctgaagtctgaggacacagccatgtattactgtgcaagagtgggagataacgac  
gactttgctatgggctactggggtcaaggaacctcagtcaccgtgtcctcaGCCAAACAACAGCCCCATCGGTCT  
45 ATCCACTGGCCCCCTGTGTGTGGAGATACAACTGGCTCCTCGGTGACcCTAGGATGCC

TGGTCAAGGGTTATTTCCCTGAGCCAGTGACCTTGACCTGGAACCTCTGGATCgCTGT  
CCAGTGGTGTGCACACCTTCCCAGCTGTCTTGACCTCTACACCCTgAGCA  
GCTCAGTGACTGTAACCTCAGCACCTGGCCCAGCCAGTCCATCACCTGCAATGTGG  
CCCACCCGGCAAGCAGCACCAAGGTGGACAAGAAAATTGAGCCCAGAGGGCCCAC  
5 AATCAAGCCCTGTCTCCATGCAAATGCCAGCACCTAACCTCTTGGGTGGACCATC  
CGTCTTCATCTTCCCTCCAAAGATCAAGGATGTACTCATGATCTCCCTGAGCCCCAT  
AGTCACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCAGATCAGCT  
GGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGAGAGGAT  
TACAACAGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCACCAGGACTGGAT  
10 GAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGACCTCCCAGCGCCCATCG  
AGAGAACCATCTCAAAACCCAAAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTG  
CCTCCACCAGAAGAAGAGATGACTAAGAAACAGGTCACTCTGACCTGCATGGTCAC  
AGACTTCATGCCTGAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGC  
TAAACTACAAGAACACTGAACCAGTCCTGGACTCTGATGGTTCTTACTTCATGTACA  
15 GCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAGAAATAGCTACTCCTGTTC  
AGTGGTCCACGAGGGTCTGCACAATCACCACACGACTAAGAGCTTCTCCCGGACTC  
CGGGTAAAgtaaacagactttgaatttgaccttctcaagttggcgggagacgtggagtccaacctggacctATGAAGTT  
GCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATCCCTGCTTCCAGCAGTgacattgtgatgtc  
acagtctccatctccctggctgtgtcagaaggagagaaggtcactttaacctgcaatccagtcagagttgtcaacagtagaacccgaa  
20 agaactacttgcttggtaccagcagaaaccagggcagcctcctaaactgttgatctactggacatccactaggggaatctggggtccctgat  
cgcttcacaggcagtggtgatgggacagatttcactctcaccatcagcagtggtgcaggctgaagacctggcagtttattactgcaacaatct  
tataataatccgtggagcttcggtggagggaccaagcttgaaataaaaCGGGCTGATGCTGCACCAACTGTATCg  
ATCTTCCCACCATCCAGTGAGCAGTTAACATCAGGAGGTGCCTCAGTCGTGTGCTTC  
TTGAACAACCTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGA  
25 ACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCT  
ACAGCATGAGCAGCACCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGC  
TATACCTGTGAGGCCACTCACAAGACATCAACTTACCCATTGTCAAaAGCTTCAAC  
AGGAATGAGTGTTAGgCGGCCGCCACTGTGCTGGATATCGTTTAAACCGCTGATCAG  
CCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTC  
30 CTTGACCCTGGAAGGTGCCACTCCCCTGTCCTTTCCTAATAAAATGAGGAAATTGC  
ATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACA  
GCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTC  
TATGG

35 > F2A Self-Cleaving Peptide (SEQ ID NO: 41)  
VKQTLNFDLLKLAGDVESNPGP

#### EQUIVALENTS

While several inventive embodiments have been described and illustrated herein, those  
40 of ordinary skill in the art will readily envision a variety of other means and/or structures for  
performing the function and/or obtaining the results and/or one or more of the advantages  
described herein, and each of such variations and/or modifications is deemed to be within the  
scope of the inventive embodiments described herein. More generally, those skilled in the art

will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, *i.e.*, elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, *i.e.*, “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); *etc.*

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, *i.e.*, the inclusion of at least one, but also

including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as  
5 indicating exclusive alternatives (*i.e.*, “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in  
10 reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified  
15 within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one,  
20 optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); *etc.*

It should also be understood that, unless clearly indicated to the contrary, in any methods  
25 claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, *i.e.*, to mean including but  
30 not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03. It should be appreciated that embodiments described in this document using an open-ended transitional phrase (*e.g.*, “comprising”) are also contemplated, in alternative embodiments, as “consisting of”  
35 and “consisting essentially of” the feature described by the open-ended transitional phrase. For

example, if the disclosure describes “a composition comprising A and B”, the disclosure also contemplates the alternative embodiments “a composition consisting of A and B” and “a composition consisting essentially of A and B”.

## CLAIMS

What is claimed is:

1. A recombinant adeno-associated virus (rAAV) vector comprising a nucleic acid sequence encoding an anti-poly-Glycine-Alanine (poly(GA)) repeat-associated non-ATG (RAN) protein antibody or antigen-binding fragment thereof, flanked by adeno-associated virus (AAV) inverted terminal repeats (ITRs).
2. The rAAV vector of claim 1, wherein the anti-poly(GA) RAN protein antibody or antigen-binding fragment thereof comprises a heavy chain variable region (VH) comprising:
  - (i) a complementarity determining region (CDR) 1 (CDR1) region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 1;
  - (ii) a CDR2 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 2; and/or
  - (iii) a CDR3 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 3.
3. The rAAV vector of claim 1 or 2, wherein the anti-poly(GA) RAN protein antibody or antigen-binding fragment comprises a light chain variable region (VL) comprising:
  - (i) a complementarity determining region (CDR) 1 (CDR1) region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 4;
  - (ii) a CDR2 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 5; and/or
  - (iii) a CDR3 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 6.
4. The rAAV vector of any one of claims 1 to 3, wherein the anti-poly(GA) RAN protein antibody or antigen-binding fragment comprises a variable heavy chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 7.
5. The rAAV vector of any one of claims 1 to 4, wherein the anti-poly(GA) RAN protein antibody or antigen-binding fragment comprises a variable light chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 12.
6. The rAAV vector of any one of claims 1 to 5, wherein the anti-poly(GA) RAN protein

antibody or antigen-binding fragment comprises a heavy chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 8 or 10.

7. The rAAV vector of any one of claims 1 to 6, wherein the anti-poly(GA) RAN protein  
5 antibody or antigen-binding fragment comprises a light chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 13 or 15.

8. The rAAV vector of claim 1, wherein the anti-poly(GA) RAN protein antibody or  
antigen-binding fragment comprises a heavy chain variable region (VH) comprising:  
10 (i) a complementarity determining region (CDR) 1 (CDR1) region comprising or  
consisting of the amino acid sequence set forth in SEQ ID NO: 20;  
(ii) a CDR2 region comprising or consisting of the amino acid sequence set forth in SEQ  
ID NO: 21; and/or  
(iii) a CDR3 region comprising or consisting of the amino acid sequence set forth in  
15 SEQ ID NO: 22.

9. The rAAV vector of claim 1 or 8, wherein the anti-poly(GA) RAN protein antibody or  
antigen-binding fragment comprises a light chain variable region (VL) comprising:  
(i) a complementarity determining region (CDR) 1 (CDR1) region comprising or  
20 consisting of the amino acid sequence set forth in SEQ ID NO: 23;  
(ii) a CDR2 region comprising or consisting of the amino acid sequence set forth in SEQ  
ID NO: 24; and/or  
(iii) a CDR3 region comprising or consisting of the amino acid sequence set forth in  
SEQ ID NO: 25.

25

10. The rAAV vector of claim 8 or 9, wherein the anti-poly(GA) RAN protein antibody or  
antigen-binding fragment comprises a variable heavy chain comprising or consisting of the  
amino acid sequence set forth in SEQ ID NO: 26.

30 11. The rAAV vector of any one of claims 8 to 10, wherein the anti-poly(GA) RAN protein  
antibody or antigen-binding fragment comprises a variable light chain comprising or consisting  
of the amino acid sequence set forth in SEQ ID NO: 31.

12. The rAAV vector of any one of claims 8 to 11, wherein the anti-poly(GA) RAN protein  
35 antibody or antigen-binding fragment comprises a heavy chain comprising or consisting of the

amino acid sequence set forth in SEQ ID NO: 27 or 29.

13. The rAAV vector of any one of claims 8 to 12, wherein the anti-poly(GA) RAN protein antibody or antigen-binding fragment comprises a light chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 32 or 34.

14. The rAAV vector of claim 1, wherein the anti-poly(GA) RAN protein antigen-binding fragment comprises a single chain variable fragment (scFv) comprising a heavy chain variable region comprising:

10 (i) a complementarity determining region (CDR) 1 (CDR1) region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 1;

(ii) a CDR2 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 2; and/or

(iii) a CDR3 region comprising or consisting of the amino acid sequence set forth in

15 SEQ ID NO: 3.

15. The rAAV vector of claim 1 or 14, wherein the anti-poly(GA) RAN protein antigen-binding fragment comprises a single chain variable fragment (scFv) comprising a light chain variable region comprising:

20 (i) a complementarity determining region (CDR) 1 (CDR1) region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 4;

(ii) a CDR2 region comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 5; and/or

(iii) a CDR3 region comprising or consisting of the amino acid sequence set forth in

25 SEQ ID NO: 6.

16. The rAAV vector of claim 14 or 15, wherein the scFv comprises a variable heavy chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 17.

30 17. The rAAV vector of any one of claims 14 to 16, wherein the scFv comprises a variable light chain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 18.

18. The rAAV vector of any one of claims 15 to 17, wherein the scFv comprises a linker molecule connecting the heavy chain variable region to the light chain variable region,

35 optionally wherein the linker molecule comprises a poly(GS) linker.

19. The rAAV vector of any one of claims 1 to 18, wherein the antibody or antigen binding fragment thereof further comprises a signal peptide.

5 20. The rAAV vector of any one of claims 1 to 19, comprising the sequence set forth in any one of SEQ ID NOs: 36-40.

21. The rAAV vector of any one of claims 1 to 20, comprising a sequence that is at least 75% identical to the sequence set forth in any one of SEQ ID NOs: 43-46.

10

22. The rAAV vector of any one of claims 1 to 21, wherein the rAAV comprises a sequence set forth in any one of SEQ ID NOs: 43-46.

23. A recombinant adeno-associated virus (rAAV) comprising:

15

- (i) the rAAV vector of any one of claims 1 to 22; and
- (ii) one or more adeno-associated virus (AAV) capsid proteins.

24. The rAAV of claim 23, wherein the one or more AAV capsid proteins comprise an AAV9 capsid protein.

20

25. The rAAV of claim 23, wherein the one or more AAV capsid proteins comprise an AAV1 capsid protein.

26. The rAAV of any one of claims 23 to 25, wherein the one or more AAV capsid proteins  
25 comprise a VP1 protein comprising an ERDRTRG peptide as set forth in SEQ ID NO: 49.

27. The rAAV of claim 26, wherein the VP1 protein comprises the amino acid sequence of SEQ ID NO: 48.

30 28. A composition comprising the rAAV vector of any one of claims 1 to 22, or the rAAV of any one of claims 23 to 27, and a pharmaceutically acceptable carrier or buffer.

29. A method for expressing an antibody or antigen-binding fragment in a subject, the method comprising administering the rAAV of any one of claims 23 to 27, to the subject.

35

30. The method of claim 29, wherein the subject is a mammal, optionally wherein the subject is a human.
31. The method of claim 29 or 30, wherein the subject expresses one or more RAN proteins.
- 5 32. The method of any one of claims 29 to 31, wherein the subject expresses a poly(GA) RAN protein.
- 10 33. The method of any one of claims 29 to 32, wherein the subject has or is suspected of having Alzheimer's disease, amyotrophic lateral sclerosis (ALS), or frontotemporal dementia.
34. The method of claim 33, wherein the subject has or is suspected of having ALS.
35. A method for reducing poly(GA) RAN protein aggregation in a subject, the method  
15 comprising administering the rAAV of any one of claims 23 to 27, to the subject.
36. The method of claim 35, wherein the subject is a mammal, optionally wherein the subject is a human.
- 20 37. The method of claim 35 or 36, wherein the subject expresses one or more RAN proteins.
38. The method of any one of claims 35 to 37, wherein the subject expresses a poly(GA) RAN protein.
- 25 39. The method of any one of claims 35 to 38, wherein the subject has or is suspected of having Alzheimer's disease, amyotrophic lateral sclerosis (ALS), or frontotemporal dementia.
40. The method of claim 39, wherein the subject has or is suspected of having ALS.
- 30 41. A method for treating a subject having amyotrophic lateral sclerosis (ALS), the method comprising administering the rAAV of any one of claims 23 to 27, to the subject.
42. The method of claim 41, wherein the subject is a human.