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(57) Abstract: Provided are recombinant adeno-associated virus (rAAV) compositions for the expression of antibodies (e.g., anti-complement component 5 (C5) antibodies) in cells, and methods of treating disorders with the same (e.g., disorders associated with C5 activity (*e.g.*, Paroxysmal Nocturnal Hemoglobinuria)). Also provided are compositions, systems and methods for making the rAAV compositions.

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# VECTORIZED ANTIBODIES AND USES THEREOF

## RELATED APPLICATIONS

**[0001]** This application claims priority to U.S. Provisional Patent Application Serial Nos. 63/075,898, filed September 9, 2020, and 63/179,990, filed April 26, 2021, the entire disclosures of which are hereby incorporated herein by reference.

## SEQUENCE LISTING

**[0002]** This application contains a sequence listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety (said ASCII copy, created on September 8, 2021, is named "404217-HMW-042WO_185407_ST25.txt" and is 252,035 bytes in size).

## BACKGROUND

**[0003]** Therapeutic antibodies represent a potent class of drugs, possessing high specificity to a target of interest. However, many antibodies require large individual doses and regular administration to achieve the desired therapeutic effect. This is especially true for antibody targets that are found at high concentrations in a patient's serum. For example, anti-complement component 5 (C5) antibodies used of the treatment of C5-mediated diseases, such as paroxysmal nocturnal hemoglobinuria (PNH), neuromyelitis optica spectrum disorder (NMOSD), and atypical hemolytic uremic syndrome (aHUS), require multiple large doses of the antibody due to the high abundance of C5 in serum.

**[0004]** Viral delivery mechanisms offer an attractive alternative to conventional antibody treatments, especially for antibody targets that are found at high concentrations in a patient's serum. In particular, a single administration of a viral vector harboring expression cassettes for antibody heavy and light chains has the potential to produce sustained therapeutic levels of an antibody in the serum of a subject, thereby bypassing the need for continual administration of high dose antibody.

**[0005]** Accordingly, there is a need in the art for improved viral vectors for the efficient and sustained expression of antibodies in a subject.

## SUMMARY

**[0006]** Provided herein are recombinant adeno-associated virus (rAAV) compositions for the expression of antibodies (e.g., anti-complement component 5 (C5) antibodies) in cells, and methods for using the same to treat disorders (e.g., disorders associated with C5 activity (*e.g.*, Paroxysmal Nocturnal Hemoglobinuria)). Also provided are compositions, systems and methods for making the rAAV compositions.

**[0007]** Accordingly, in one aspect, the disclosure provides a recombinant adeno-associated virus (rAAV) genome comprising:

(a) a first expression cassette comprising, from 5′ to 3′,

a first liver-specific transcriptional regulatory element,

a first coding sequence encoding a first polypeptide comprising an antibody heavy chain operably linked to a first signal sequence, and

a first polyadenylation sequence; and

(b) a second expression cassette comprising, from 5′ to 3′,

a second liver-specific transcriptional regulatory element,

a second coding sequence encoding a second polypeptide comprising an antibody light chain operably linked to a second signal sequence, and

a second polyadenylation sequence,

wherein expression of the first and second coding sequences produces an antibody comprising the antibody heavy chain and the antibody light chain.

**[0008]** In certain embodiments, the first and/or second transcriptional regulatory element comprise a promoter element selected from the group consisting of human albumin promoter, a human transthyretin (TTR) promoter, a human thyroxine binding globulin (TBG) promoter, a human ApoH promoter, a human SERPINA1 (hAAT) promoter, and a hepatic specific regulatory module thereof, such as a human ApoE/C-I hepatic control region (HCR) 1 or 2.

**[0009]** In certain embodiments, the first and/or second transcriptional regulatory element comprise a promoter element comprising a nucleic acid sequence at least 90% identical to a sequence selected from the group consisting of SEQ ID NO: 25, 27, 66, 68, 69, 116, and 117.

**[0010]** In certain embodiments, the transcriptional regulatory element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 27. In certain embodiments, the transcriptional regulatory element comprises the nucleotide sequence set forth in SEQ ID NO: 27. In certain embodiments, the nucleotide sequence of the

transcriptional regulatory element consists of the nucleotide sequence set forth in SEQ ID NO: 27.

**[0011]** In certain embodiments, the transcriptional regulatory element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 67. In certain embodiments, the transcriptional regulatory element comprises the nucleotide sequence set forth in SEQ ID NO: 67. In certain embodiments, the nucleotide sequence of the transcriptional regulatory element consists of the nucleotide sequence set forth in SEQ ID NO: 67.

**[0012]** In certain embodiments, the first and/or second expression cassette further comprise an intron element positioned 5′ to the first and/or second coding sequence and 3′ to the transcriptional regulatory element.

**[0013]** In certain embodiments, the intron element is an exogenous intron element, optionally wherein the exogenous intron element is an SV40 intron element or a minute virus of mouse (MVM) intron element.

**[0014]** In certain embodiments, the SV40 intron element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 29. In certain embodiments, the SV40 intron element comprises the nucleotide sequence set forth in SEQ ID NO: 29. In certain embodiments, the nucleotide sequence of the SV40 intron element consists of the nucleotide sequence set forth in SEQ ID NO: 29.

**[0015]** In certain embodiments, the MVM intron element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 30. In certain embodiments, the MVM intron element comprises the nucleotide sequence set forth in SEQ ID NO: 30. In certain embodiments, the nucleotide sequence of the MVM intron element consists of the nucleotide sequence set forth in SEQ ID NO: 30.

**[0016]** In certain embodiments, the first and second transcriptional regulatory element are identical.

**[0017]** In certain embodiments, the first transcriptional regulatory element comprises an HCR 1 element, a hAAT promoter, and an SV40 intron element, and the second transcriptional regulatory element comprises a SERPINA1 hepatic specific regulatory module, a TTR promoter, and an MVM intron element.

**[0018]** In certain embodiments, the first transcriptional regulatory element comprises the nucleic acid sequence of SEQ ID NO: 50 and the second transcriptional regulatory element comprises the nucleic acid sequence of SEQ ID NO: 43.

**[0019]** In certain embodiments, the first and/or second expression cassette further comprise a polyadenylation sequence 3′ to the first and/or second coding sequence.

**[0020]** In certain embodiments, the polyadenylation sequence is an exogenous polyadenylation sequence, optionally wherein the exogenous polyadenylation sequence is an SV40 polyadenylation sequence, or a bovine growth hormone (BGH) polyadenylation sequence.

**[0021]** In certain embodiments, the SV40 polyadenylation sequence comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 31. In certain embodiments, the SV40 polyadenylation sequence comprises the nucleotide sequence set forth in SEQ ID NO: 31. In certain embodiments, the nucleotide sequence of the SV40 polyadenylation sequence consists of the nucleotide sequence set forth in SEQ ID NO: 31.

**[0022]** In certain embodiments, the BGH polyadenylation sequence comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 33. In certain embodiments, the BGH polyadenylation sequence comprises the nucleotide sequence set forth in SEQ ID NO: 33. In certain embodiments, the nucleotide sequence of the BGH polyadenylation sequence consists of the nucleotide sequence set forth in SEQ ID NO: 33.

**[0023]** In certain embodiments, the first and second expression cassette comprise identical polyadenylation sequences.

**[0024]** In certain embodiments, the first expression cassette comprises the SV40 polyadenylation sequence. In certain embodiments, the second expression cassette comprises the BGH polyadenylation sequence.

**[0025]** In certain embodiments, the first polyadenylation sequence comprises the nucleic acid sequence of SEQ ID NO: 31 and the second polyadenylation sequence comprises the nucleic acid sequence of SEQ ID NO: 33.

**[0026]** In certain embodiments, the first and second expression cassettes are in the same orientation in the rAAV genome. In certain embodiments, the first and second expression cassettes are in opposite orientations in the rAAV genome.

**[0027]** In certain embodiments, the first and second expression cassettes are in opposite orientations, with the first and second polyadenylation sequences distally positioned in the rAAV genome.

**[0028]** In certain embodiments, the rAAV genome further comprises a stuffer sequence interposed between the first and second transcriptional regulatory elements.

**[0029]** In certain embodiments, the stuffer sequence comprises a beta globin polyadenylation sequence. In certain embodiments, the beta globin polyadenylation sequence comprises the nucleic acid sequence of SEQ ID NO: 51.

**[0030]** In certain embodiments, the rAAV genome comprises from 5′ to 3′: (a) the first polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 33; (b) the first coding sequence; (c) the first liver-specific transcriptional regulatory element comprising the nucleic acid sequence of SEQ ID NO: 27; (d) a stuffer sequence comprising the nucleic acid sequence of SEQ ID NO: 51; (e) the second liver-specific transcriptional regulatory element comprising the nucleic acid sequence of SEQ ID NO: 67; (f) the second coding sequence; (g) the second transcriptional polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 31.

**[0031]** In certain embodiments, the rAAV genome comprises from 5′ to 3′: the reverse complement of the first expression cassette; a stuffer sequence; and the second expression cassette.

**[0032]** In certain embodiments, the rAAV genome comprises: (a) the first expression cassette comprises, from 5′ to 3′: a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 27, the first coding sequence, a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 33; (b) the stuffer sequence comprising a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and (c) the second expression cassette comprising, from 5′ to 3′, a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 67, the second coding sequence, a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 31.

**[0033]** In certain embodiments, the rAAV genome comprises: (a) the first expression cassette comprises, from 5′ to 3′: a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 67, the first coding sequence, a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 31; (b) the stuffer sequence comprising a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and (c) the second expression cassette comprising, from 5′ to 3′, a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 27, the second coding sequence, a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 33.

**[0034]** In certain embodiments, the rAAV genome comprises: (a) the first expression cassette comprises, from 5′ to 3′: a nucleotide sequence at least 90% identical to the nucleotide

sequence set forth in SEQ ID NO: 25, a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 26, the first coding sequence, the first polyadenylation sequence; (b) the stuffer sequence comprising a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and (c) the second expression cassette comprising, from 5′ to 3′, a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 119, a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 45, the second coding sequence, a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 31.

**[0035]** In certain embodiments, the rAAV genome comprises: (a) the first expression cassette comprises, from 5′ to 3′: a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 119, a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 45, the first coding sequence, a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 31; (b) the stuffer sequence comprising a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and (c) the second expression cassette comprising, from 5′ to 3′, a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 25, a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 26, the second coding sequence, the first polyadenylation sequence.

**[0036]** In certain embodiments, the rAAV genome comprises: (a) the first expression cassette comprises, from 5′ to 3′: the nucleotide sequence set forth in SEQ ID NO: 27, the first coding sequence, the nucleotide sequence set forth in SEQ ID NO: 33; (b) the stuffer sequence comprising the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and (c) the second expression cassette comprising, from 5′ to 3′, the nucleotide sequence set forth in SEQ ID NO: 67, the second coding sequence, the nucleotide sequence set forth in SEQ ID NO: 31.

**[0037]** In certain embodiments, the rAAV genome comprises: (a) the first expression cassette comprises, from 5′ to 3′: the nucleotide sequence set forth in SEQ ID NO: 67, the first coding sequence, the nucleotide sequence set forth in SEQ ID NO: 31; (b) the stuffer sequence comprising the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and (c) the second expression cassette comprising, from 5′ to 3′, the nucleotide sequence set forth in SEQ ID NO: 27, the second coding sequence, the nucleotide sequence set forth in SEQ ID NO: 33.

**[0038]** In certain embodiments, the rAAV genome comprises: (a) the first expression cassette comprises, from 5′ to 3′: the nucleotide sequence set forth in SEQ ID NO: 25, the nucleotide sequence set forth in SEQ ID NO: 26, the first coding sequence, the first polyadenylation sequence; (b) the stuffer sequence comprising the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and (c) the second expression cassette comprising, from 5′ to 3′, the nucleotide sequence set forth in SEQ ID NO: 119, the nucleotide sequence set forth in SEQ ID NO: 45, the second coding sequence, the nucleotide sequence set forth in SEQ ID NO: 31.

**[0039]** In certain embodiments, the rAAV genome comprises: (a) the first expression cassette comprises, from 5′ to 3′: the nucleotide sequence set forth in SEQ ID NO: 119, the nucleotide sequence set forth in SEQ ID NO: 45, the first coding sequence, the nucleotide sequence set forth in SEQ ID NO: 31; (b) the stuffer sequence comprising the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and (c) the second expression cassette comprising, from 5′ to 3′, the nucleotide sequence set forth in SEQ ID NO: 25, the nucleotide sequence set forth in SEQ ID NO: 26, the second coding sequence, the first polyadenylation sequence.

**[0040]** In one aspect, the disclosure provides an rAAV genome comprising a bicistronic expression cassette comprising, from 5′ to 3′:

(a) a liver-specific transcriptional regulatory element; a first coding sequence encoding a first polypeptide comprising an antibody heavy chain operably linked to a first signal sequence; a ribosomal skipping sequence encoding a ribosomal skipping peptide; a second coding sequence encoding a second polypeptide comprising an antibody light chain operably linked to a second signal sequence; and a polyadenylation sequence, or

(b) a liver-specific transcriptional regulatory element; a second coding sequence encoding a second polypeptide comprising an antibody light chain operably linked to a second signal sequence; a ribosomal skipping sequence encoding a ribosomal skipping peptide; a first coding sequence encoding a first polypeptide comprising an antibody heavy chain operably linked to a first signal sequence; and a polyadenylation sequence,

wherein expression of the bicistronic expression cassette produces an antibody comprising the antibody heavy chain and the antibody light chain.

**[0041]** In certain embodiments, the transcriptional regulatory element comprises a promoter element selected from the group consisting of human albumin promoter, a human transthyretin (TTR) promoter, the human thyroxine binding globulin (TBG) promoter, a human

ApoH promoter, a human SERPINA1 (hAAT) promoter, and a hepatic specific regulatory module thereof, such as a human ApoE/C-I hepatic control region (HCR) 1 or 2.

**[0042]** In certain embodiments, the transcriptional regulatory element comprises a promoter element comprising a nucleic acid sequence at least 90% identical to a sequence selected from the group consisting of SEQ ID NO: 25, 27, 66, 68, 69, 116, and 117.

**[0043]** In certain embodiments, the transcriptional regulatory element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 27. In certain embodiments, the transcriptional regulatory element comprises the nucleotide sequence set forth in SEQ ID NO: 27. In certain embodiments, the nucleotide sequence of the transcriptional regulatory element consists of the nucleotide sequence set forth in SEQ ID NO: 27.

**[0044]** In certain embodiments, the transcriptional regulatory element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 67. In certain embodiments, the transcriptional regulatory element comprises the nucleotide sequence set forth in SEQ ID NO: 67. In certain embodiments, the nucleotide sequence of the transcriptional regulatory element consists of the nucleotide sequence set forth in SEQ ID NO: 67.

**[0045]** In certain embodiments, the bicistronic expression cassette further comprises an intron element positioned 5′ to the first and/or second coding sequence and 3′ to the transcriptional regulatory element.

**[0046]** In certain embodiments, the intron element is an exogenous intron element, optionally wherein the exogenous intron element is an SV40 intron element or a minute virus of mouse (MVM) intron element.

**[0047]** In certain embodiments, the SV40 intron element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 29. In certain embodiments, the SV40 intron element comprises the nucleotide sequence set forth in SEQ ID NO: 29. In certain embodiments, the nucleotide sequence of the SV40 intron element consists of the nucleotide sequence set forth in SEQ ID NO: 29.

**[0048]** In certain embodiments, the MVM intron element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 30. In certain embodiments, the MVM intron element comprises the nucleotide sequence set forth in SEQ ID NO: 30. In certain embodiments, the nucleotide sequence of the MVM intron element consists of the nucleotide sequence set forth in SEQ ID NO: 30.

**[0049]** In certain embodiments, the transcriptional regulatory element comprises:

a) an HCR 1 element, a hAAT promoter, and an SV40 intron element; or

b) a SERPINA1 hepatic specific regulatory module, a TTR promoter, and an MVM intron element.

**[0050]** In certain embodiments, the transcriptional regulatory element comprises the nucleic acid sequence of SEQ ID NO: 50, or the nucleic acid sequence of SEQ ID NO: 43.

**[0051]** In certain embodiments, the polyadenylation sequence is an exogenous polyadenylation sequence, optionally wherein the exogenous polyadenylation sequence is an SV40 polyadenylation sequence, or a bovine growth hormone (BGH) polyadenylation sequence.

**[0052]** In certain embodiments, the SV40 polyadenylation sequence comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 31. In certain embodiments, the SV40 polyadenylation sequence comprises the nucleotide sequence set forth in SEQ ID NO: 31. In certain embodiments, the nucleotide sequence of the SV40 polyadenylation sequence consists of the nucleotide sequence set forth in SEQ ID NO: 31.

**[0053]** In certain embodiments, the BGH polyadenylation sequence comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 33. In certain embodiments, the BGH polyadenylation sequence comprises the nucleotide sequence set forth in SEQ ID NO: 33. In certain embodiments, the nucleotide sequence of the BGH polyadenylation sequence consists of the nucleotide sequence set forth in SEQ ID NO: 33.

**[0054]** In certain embodiments, the first and/or second signal sequence is a naturally occurring signal sequence. In certain embodiments, the first and/or second signal sequence is an antibody signal sequence, optionally a human IgG2 or IgK signal sequence. In certain embodiments, the first and/or second signal sequence is a non-naturally occurring signal sequence. In certain embodiments, the first and/or second signal sequence comprises the amino acid sequence of SEQ ID NO: 80. In certain embodiments, the first and/or second signal sequence comprises the amino acid sequence of SEQ ID NO: 81. In certain embodiments, the first signal sequence comprises the amino acid sequence of SEQ ID NO: 80 and the second signal sequence comprises the amino acid sequence of SEQ ID NO: 81. In certain embodiments, the first and/or second coding sequence comprises any one of the nucleic acid sequences set forth in SEQ ID NO: 23, 96, 102, or 108. In certain embodiments, the first and/or second coding sequence comprises any one of the nucleic acid sequences set forth in SEQ ID NO: 24, 99, 105, 111, or 130. In certain embodiments, the first coding sequence comprises

any one of the nucleic acid sequences set forth in SEQ ID NO: 23, 96, 102, or 108 and the second coding sequence comprises any one of the nucleic acid sequences set forth in SEQ ID NO: 24, 99, 105, 111, or 130.

**[0055]** In certain embodiments, the antibody specifically binds to complement C5.

**[0056]** In certain embodiments, the antibody heavy chain comprises the amino acid sequence of SEQ ID NO: 64. In certain embodiments, the antibody heavy chain comprises the amino acid sequence of SEQ ID NO: 82. In certain embodiments, the antibody light chain comprises the amino acid sequence of SEQ ID NO: 77.

**[0057]** In certain embodiments, the first and/or second coding sequence has been optimized for expression in human cells.

**[0058]** In certain embodiments, the first coding sequence comprises any one of the nucleic acid sequences set forth in SEQ ID NO: 52, 113, 114, or 115.

**[0059]** In certain embodiments, the first coding sequence comprises any one of the nucleic acid sequences set forth in SEQ ID NO: 83, 94, 95, 101, or 107.

**[0060]** In certain embodiments, the second coding sequence comprises any one of the nucleic acid sequences set forth in SEQ ID NO: 53, 98, 104, 110, or 131.

**[0061]** In certain embodiments, the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 53.

**[0062]** In certain embodiments, the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 63.

**[0063]** In certain embodiments, the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 98.

**[0064]** In certain embodiments, the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 99.

**[0065]** In certain embodiments, the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101,

102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 100.

**[0066]** In certain embodiments, the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 104.

**[0067]** In certain embodiments, the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 105.

**[0068]** In certain embodiments, the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 106.

**[0069]** In certain embodiments, the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 110.

**[0070]** In certain embodiments, the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 111.

**[0071]** In certain embodiments, the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 112.

**[0072]** In certain embodiments, the rAAV genome is a single stranded rAAV genome.

**[0073]** In certain embodiments, the rAAV genome is a self-complementary rAAV genome.

**[0074]** In certain embodiments, the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 84. In certain embodiments, the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 85. In certain embodiments, the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 86. In certain embodiments, the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 87.

**[0075]** In certain embodiments, the rAAV genome further comprises a 5′ inverted terminal repeat (5′ ITR) nucleotide sequence 5′ to the first polyadenylation sequence, and a 3′ inverted terminal repeat (3′ ITR) nucleotide sequence 3′ the second polyadenylation sequence.

**[0076]** In certain embodiments, the 5′ ITR nucleotide sequence is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence set forth in SEQ ID NO: 14, and/or the 3′ ITR nucleotide sequence is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence set forth in SEQ ID NO: 18.

**[0077]** In certain embodiments, the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 88. In certain embodiments, the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 89. In certain embodiments, the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 90. In certain embodiments, the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 91.

**[0078]** In another aspect, the disclosure provides a recombinant adeno-associated virus (rAAV) comprising:

(a) an AAV capsid comprising an AAV capsid protein; and

(b) an rAAV genome of any of the above embodiments.

**[0079]** In certain embodiments, the capsid protein is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, and AAV9.

**[0080]** In certain embodiments, the AAV capsid protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of amino acids 203-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

**[0081]** In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 206 of SEQ ID NO: 16 is C; the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H; the amino acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q; the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A; the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N; the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S; the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I; the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 590 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G or Y; the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M; the amino acid in the capsid protein corresponding

to amino acid 687 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K; the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C; or, the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G.

**[0082]** In certain embodiments,

(a) the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G, and the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G;

(b) the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H, the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M;

(c) the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R;

(d) the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A, and the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; or

(e) the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C.

**[0083]** In certain embodiments, the capsid protein comprises the amino acid sequence of amino acids 203-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

**[0084]** In certain embodiments, the AAV capsid protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of amino acids 138-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

**[0085]** In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 151 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 160 of SEQ ID NO: 16 is D; the amino acid in the capsid protein corresponding to amino acid 206 of SEQ ID NO: 16 is C; the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H; the amino acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q; the amino acid in the capsid protein corresponding to

amino acid 346 of SEQ ID NO: 16 is A; the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N; the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S; the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I; the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 590 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G or Y; the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M; the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K; the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C; or, the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G.

**[0086]** In certain embodiments,

(a) the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G, and the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G;

(b) the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H, the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M;

(c) the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R;

(d) the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A, and the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; or

(e) the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C.

**[0087]** In certain embodiments, the capsid protein comprises the amino acid sequence of amino acids 138-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 15, 16, or 17.

**[0088]** In certain embodiments, the AAV capsid protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of amino acids 1-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

**[0089]** In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 2 of SEQ ID NO: 16 is T; the amino acid in the capsid protein corresponding to amino acid 65 of SEQ ID NO: 16 is I; the amino acid in the capsid protein corresponding to amino acid 68 of SEQ ID NO: 16 is V; the amino acid in the capsid protein corresponding to amino acid 77 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 119 of SEQ ID NO: 16 is L; the amino acid in the capsid protein corresponding to amino acid 151 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 160 of SEQ ID NO: 16 is D; the amino acid in the capsid protein corresponding to amino acid 206 of SEQ ID NO: 16 is C; the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H; the amino acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q; the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A; the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N; the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S; the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I; the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 590 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G or Y; the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M; the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K; the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C; or, the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G.

**[0090]** In certain embodiments,

(a) the amino acid in the capsid protein corresponding to amino acid 2 of SEQ ID NO: 16 is T, and the amino acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q;

(b) the amino acid in the capsid protein corresponding to amino acid 65 of SEQ ID NO: 16 is I, and the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is Y;

(c) the amino acid in the capsid protein corresponding to amino acid 77 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K;

(d) the amino acid in the capsid protein corresponding to amino acid 119 of SEQ ID NO: 16 is L, and the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S;

(e) the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G, and the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G;

(f) the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H, the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M;

(g) the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R;

(h) the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A, and the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; or

(i) the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C.

**[0091]** In certain embodiments, the capsid protein comprises the amino acid sequence of amino acids 1-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

**[0092]** In one aspect, the disclosure provides a polynucleotide comprising the nucleic acid sequence set forth in SEQ ID NOs: 85-93.

**[0093]** In one aspect, the disclosure provides a pharmaceutical composition comprising an rAAV described above or the polynucleotide described above.

**[0094]** In one aspect, the disclosure provides a packaging system for preparation of an rAAV, wherein the packaging system comprises:

(a) a first nucleotide sequence encoding one or more AAV Rep proteins;

(b) a second nucleotide sequence encoding a capsid protein of the rAAV described above; and

(c) a third nucleotide sequence comprising an rAAV genome sequence of the rAAV described above.

**[0095]** In certain embodiments, the packaging system comprises a first vector comprising the first nucleotide sequence and the second nucleotide sequence, and a second vector comprising the third nucleotide sequence.

**[0096]** In certain embodiments, the packaging system further comprises a fourth nucleotide sequence comprising one or more helper virus genes. In certain embodiments, the fourth nucleotide sequence is comprised within a third vector. In certain embodiments, the fourth nucleotide sequence comprises one or more genes from a virus selected from the group consisting of adenovirus, herpes virus, vaccinia virus, and cytomegalovirus (CMV).

**[0097]** In certain embodiments, the first vector, second vector, and/or the third vector is a plasmid.

**[0098]** In one aspect, the disclosure provides a method for recombinant preparation of an rAAV, the method comprising introducing the packaging system described above into a cell under conditions whereby the rAAV is produced.

**[0099]** In another aspect, the disclosure provides the rAAV described above, the pharmaceutical composition described above, or the polynucleotide described above, for use as a medicament.

**[00100]** In another aspect, the disclosure provides the rAAV described above, the pharmaceutical composition described above, or the polynucleotide described above, for use in the treatment of complement C5-associated disease.

**[00101]** In another aspect, the disclosure provides the rAAV described above, the pharmaceutical composition described above, or the polynucleotide described above, for use in a method of treating a subject having a complement C5-associated disease, the method comprising administering to the subject an effective amount of the rAAV, the pharmaceutical composition, or the polynucleotide.

**[00102]** In one aspect, the disclosure provides a method of producing an antibody in a subject, the method comprising administering to the subject the pharmaceutical composition of described above.

**[00103]** In certain embodiments, the pharmaceutical composition is administered intravenously.

**[00104]** In one aspect, the disclosure provides a method of treating a complement C5-associated disease in a subject in need thereof, the method comprising administering to the

subject an effective amount of the rAAV described above, the pharmaceutical composition described above or the polynucleotide described above.

**[00105]** In certain embodiments, the complement C5-associated disease is selected from the group consisting of geographic atrophy (GA), Guillain-Barré syndrome, myasthenia gravis, systemic lupus erythematous (SLE) nephritis, proliferative nephritis, asthma, rheumatoid arthritis, sepsis, paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), and age-related macular degeneration (AMD).

**[00106]** In certain embodiments, the rAAV, the pharmaceutical composition, or the polynucleotide is administered intravenously.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[00107]** **FIG. 1** depicts vector maps of the expression cassettes of rAAV vectors C5Ab01, C5Ab02, C5Ab03 and C5Ab04.

**[00108]** **FIG. 2A – FIG. 2I** depict graphs showing the anti-C5 antibody concentration in the serum of NOD SCID mice receiving anti-C5 antibody expressing vectors (C5Ab02, C5Ab03, and C5Ab04) packaged in the AAVHSC13, AAVHSC15, or AAVHSC17 capsid. **FIG. 2A** depicts a graph showing the anti-C5 antibody concentration in the serum of mice receiving vector C5Ab04 packaged in the AAVHSC13 or AAVHSC17 capsid at a dose of 1e13 vgs/kg. Data for male and female mice were segregated and multiple serum samples were taken over a period of 23 weeks. **FIG. 2B** depicts a graph showing the results in FIG. 2A with the Y-axis in a logarithmic scale. **FIG. 2C** depicts a graph showing the anti-C5 antibody concentration in the serum of mice receiving vector C5Ab02 packaged in the AAVHSC17 capsid at a dose of 1e13 vgs/kg. Data for male and female mice were segregated and multiple serum samples were taken over a period of 16 weeks. **FIG. 2D** depicts a graph showing the results in FIG. 2C with the Y-axis in a logarithmic scale. **FIG. 2E** depicts a graph showing the anti-C5 antibody concentration in the serum of mice receiving vector C5Ab02, C5Ab03, or C5Ab04, each packaged in the AAVHSC15 or AAVHSC17 capsid at a dose of 1e13 vgs/kg. Data for male mice is shown and multiple serum samples were taken over a period of 16 weeks. **FIG. 2F** shows the results in FIG. 2E depicted in a line graph format, and **FIG. 2G** shows the results in FIG. 2E depicted in a line graph format with the Y-axis in a logarithmic scale. **FIG. 2H** depicts a graph showing the anti-C5 antibody concentration in the serum of mice receiving vector C5Ab04 packaged in the AAVHSC17 capsid at 5 doses, 5e11 vgs/kg, 5e12 vgs/kg, 1.4e13 vgs/kg, 4.4e13 vgs/kg, and 1.8e14 vgs/kg. Data for male mice is shown and multiple

serum samples were taken over a period of 13 weeks. **FIG. 2I** depicts a graph showing the results in FIG. 2H with the Y-axis in a logarithmic scale.

**[00109]** **FIG. 3A – FIG. 3C** depict graphs showing anti-C5 antibody concentrations in the serum of NOD SCID male mice receiving anti-C5 antibody expressing vectors. The data is derived from FIG. 2 above and ordered to compare vectors C5Ab02, C5Ab03, or C5Ab04 packaged in the AAVHSC13, AAVHSC15, or AAVHSC17 capsid. **FIG. 3A** depicts a graph showing the anti-C5 antibody concentration in the serum of mice receiving vector C5Ab04 packaged in the AAVHSC13 capsid at a dose of 1e13 vgs/kg. **FIG. 3B** depicts a graph showing the anti-C5 antibody concentration in the serum of mice receiving vector C5Ab02, C5Ab03, or C5Ab04 packaged in the AAVHSC15 capsid at a dose of 1e13 vgs/kg. **FIG. 3C** depicts a graph showing the anti-C5 antibody concentration in the serum of mice receiving vector C5Ab02, C5Ab03, or C5Ab04 packaged in the AAVHSC17 capsid at a dose of 1e13 vgs/kg.

**[00110]** **FIG. 4A** depicts a graph comparing the predicted anti-C5 antibody concentrations in PNH patients receiving chronic maintenance therapy with anti-C5 antibodies eculizumab and ravulizumab to anti-C5 antibody concentrations measured in NOD SCID male and female mice using either the AAVHSC13 or AAVHSC17 capsid (data from FIG. 2B). **FIG. 4B** depicts a graph comparing the predicted anti-C5 antibody concentrations in PNH patients receiving anti-C5 antibodies eculizumab and ravulizumab to anti-C5 antibody concentrations measured in NOD SCID and HuLiv mice using the AAVHSC17 capsid (representative data from FIGs. 2A, 2I, and 8B).

**[00111]** **FIG. 5** depicts a graph of % hemolysis of activated sheep red blood cells (RBCs) at various concentrations of anti-C5 antibody in an *ex vivo* hemolysis assay. An anti-C5 control antibody was compared against serum obtained from mice treated with AAVHSC13-packaged C5Ab04 and AAVHSC17-packaged C5Ab04.

**[00112]** **FIG. 6A** depicts a graph of serum antibody concentration and **FIG. 6B** depicts a graph of % hemolysis of activated sheep RBCs in an *ex vivo* hemolysis assay. Negative control mouse serum was compared against serum obtained from mice treated with AAVHSC13-packaged C5Ab04 and AAVHSC17-packaged C5Ab04, each at a dose of 1e13 vgs/kg, at 1, 3, 5, 7, and 9 weeks after administration. **FIG. 6C** depicts a graph showing the results in FIG. 6B with % hemolysis determined from serum samples obtained out to 19 weeks post-administration, and presented in a line graph. **FIG. 6D** depicts a graph of % hemolysis of activated sheep RBCs in an *ex vivo* hemolysis assay performed using serum obtained from mice treated with AAVHSC17-packaged C5Ab02 at a dose of 1e13 vgs/kg. **FIG. 6E** depicts a graph of % hemolysis of activated sheep RBCs in an *ex vivo* hemolysis assay performed using

serum obtained from mice treated with AAVHSC15 or AAVHSC17-packaged C5Ab02, C5Ab03, or C5Ab04, each at a dose of 1e13 vgs/kg. **FIG. 6F** depicts a graph of % hemolysis of activated sheep RBCs in an *ex vivo* hemolysis assay performed using serum obtained from mice treated with AAVHSC17-packaged C5Ab04 at doses of 5e11 vgs/kg, 5e12 vgs/kg, 1.4e13 vgs/kg, 4.4e13 vgs/kg, and 1.8e14 vgs/kg. In FIGs. 6A-6F, data for male and female mice was segregated, and data in FIGs. 6E and 6F were from male mice.

**[00113]** **FIG. 7A – FIG. 7B** depict graphs comparing: the level of human C5 in the serum of FRGKO humanized liver mice (referred hereafter as HuLiv, Yecuris) in either C57Bl/6 (FRGC57) or NOD (FRGNOD) background to the level of human C5 found in human serum (FIG. 7A); and level of mouse C5 in the serum of HuLiv mice in C57Bl6 or NOD background (FIG. 7B). FRGC57_Donor A represents the HuLiv mouse with hepatocytes from patient donor A (n = 3). FRGC57_Donor B represents the HuLiv mouse with hepatocytes from patient donor B (n = 3). NOD_Donor A represents the HuLiv mouse from a congenic NOD mouse with hepatocytes from patient donor A (n = 9).

**[00114]** **FIG. 8A** depicts a graph of serum antibody concentration of anti-C5 antibodies in HuLiv mice administered 100 µg of an anti-C5 antibody (biosimilar), or C5Ab04 packaged in the AAVHSC17 capsid at a dose of 1e13 vgs/kg or 1e14 vgs/kg, in each case at 0, 1, 3, and 5 weeks after administration. In FIG. 8A, PB indicates pre-bleed. **FIG. 8B** shows the data depicted in FIG. 8A with serum antibody concentration determined out to week 11 after administration, presented in a line graph. **FIG. 8C** depicts a graph of % hemolysis of activated sheep RBCs in an *ex vivo* hemolysis assay performed using serum obtained from mice administered 100 µg of an anti-C5 antibody (biosimilar), or C5Ab04 packaged in the AAVHSC17 capsid at a dose of 1e13 vgs/kg or 1e14 vgs/kg. **FIG. 8D** depicts a graph showing the level of mouse C5 detected in serum obtained from mice administered a single dose of 100 µg of an anti-C5 antibody (biosimilar), or C5Ab04 packaged in the AAVHSC17 capsid at a dose of 1e13 vgs/kg or 1e14 vgs/kg. **FIG. 9A – FIG. 9B** depict a western blot (FIG. 9A) and human IgG ELISA data (FIG. 9B) of the level of human C5 in the culture media of primary human and mouse hepatocytes that were transduced with C5Ab02, C5Ab03, or C5Ab04, packaged in AAVHSC15 or AAVHSC17 capsids.

## DETAILED DESCRIPTION

**[00115]** Provided herein are rAAV genomes and rAAV for the expression of antibodies (e.g., anti-C5 antibodies) in cells (e.g., liver cells), and methods for using the same to treat

disorders with the same (e.g., disorders associated with C5 activity (*e.g.*, Paroxysmal Nocturnal Hemoglobinuria)). Also provided are nucleic acids, vectors, packaging systems, and methods for making the rAAV.

## I. Definitions

**[00116]** As used herein, the terms "recombinant adeno-associated virus" or "rAAV" refers to an AAV comprising a genome lacking functional rep and cap genes.

**[00117]** As used herein, the term "rAAV genome" refers to a nucleic acid molecule (*e.g.*, DNA and/or RNA) comprising the genome sequence of an rAAV. The skilled artisan will appreciate that where an rAAV genome comprises a transgene (*e.g.*, an antibody heavy chain or light chain coding sequence operably linked to a transcriptional regulatory element), the rAAV genome can be in the sense or antisense orientation relative to the direction of transcription of the transgene.

**[00118]** As used herein, the term "AAV capsid protein" refers to an AAV VP1, VP2, or VP3 capsid protein.

**[00119]** As used herein, the "percentage identity" between two nucleotide sequences or between two amino acid sequences is calculated by multiplying the number of matches between the pair of aligned sequences by 100, and dividing by the length of the aligned region, including internal gaps. Identity scoring only counts perfect matches, and does not consider the degree of similarity of amino acids to one another. Note that only internal gaps are included in the length, not gaps at the sequence ends.

**[00120]** As used herein, the term "coding sequence" refers to the portion of a complementary DNA (cDNA) that encodes a polypeptide, starting at the start codon and ending at the stop codon. A gene may have one or more coding sequences due to alternative splicing, alternative translation initiation, and variation within the population. A coding sequence may either be wild-type, silently-altered, or intron-inserted. Exemplary anti-C5 heavy chain coding sequences are set forth in SEQ ID NOs: 52 and 83. An exemplary anti-C5 light chain coding sequence is set forth in SEQ ID NO: 53. A coding sequence may be codon optimized. Codon optimization may be performed to enhance expression of the coding sequence in a desired host cell, such as a human cell. Exemplary codon optimized anti-C5 heavy chain coding sequences are set forth in SEQ ID NOs: 94, 95, 101, 107, 113, 114, and 115. Exemplary codon optimized anti-C5 light chain coding sequence is set forth in SEQ ID NO: 98, 104, 110, or 131.

**[00121]** In certain embodiments, two or more coding sequences (e.g., an antibody heavy chain coding sequence and an antibody light chain coding sequence) can be separated by a

nucleotide sequence encoding a peptide cleavage sequence, such as the 2A peptide ribosomal skipping elements. Exemplary 2A peptide cleavage sequences are set forth in SEQ ID NO: 28 or 125 (T2A peptide cleavage sequences), or 128 (P2A peptide cleavage sequence). The 2A peptide cleavage sequences may further comprise a furin cleavage sequence and linker. Exemplary 2A peptide cleavage sequences with the furin cleavage sequence and linker are set forth in SEQ ID NO: 127 or 129.

**[00122]** As used herein, the term "polyadenylation sequence" refers to a DNA sequence that when transcribed into RNA constitutes a polyadenylation signal sequence. The polyadenylation sequence can be native or exogenous. The exogenous polyadenylation sequence can be a mammalian or a viral polyadenylation sequence (e.g., a bovine growth hormone polyadenylation sequence or an SV40 polyadenylation sequence).

**[00123]** As used herein, the term "intron element" refers to a cis-acting nucleotide sequence, for example, a DNA sequence, that regulates (e.g., controls, increases, or reduces) expression of a transgene. In certain embodiments, an intron element is a modified intron, e.g., a synthetic intron sequence. In certain embodiments, an intron element is an exogenous intron element and is derived from an intron exogenous to the transgene it may regulate. In certain embodiments, an intron element comprises a modified splice acceptor and/or splice donor resulting in more robust splicing activity. While not wishing to be bound by theory, it is hypothesized that introns can increase transgene expression, for example, by reducing transcriptional silencing and enhancing mRNA export from the nucleus to the cytoplasm. A skilled worker will appreciate that synthetic intron sequences can be designed to mediate RNA splicing by introducing any consensus splicing motifs known in the art (e.g., in Sibley et al. (2016) Nature Reviews Genetics, 17, 407-21, which is incorporated by reference herein in its entirety). Exemplary intron sequences are provided in Lu et al. (2013) Molecular Therapy 21(5): 954-63, and Lu et al. (2017) Hum. Gene Ther. 28(1): 125-34, which are incorporated by reference herein in their entirety.

**[00124]** As used herein, the term "silently-altered" refers to alteration of a coding sequence of a gene (e.g., by nucleotide substitution) without changing the amino acid sequence of the polypeptide encoded by the coding sequence or stuffer-inserted coding sequence. Such silent alteration is advantageous in that it may increase the translation efficiency of a coding sequence, and/or prevent recombination with a corresponding sequence of an endogenous gene when a coding sequence is transduced into a cell.

**[00125]** As used herein, the term "transcriptional regulatory element" or "TRE" refers to a cis-acting nucleotide sequence, for example, a DNA sequence, that regulates (e.g., controls,

increases, or reduces) transcription of an operably linked nucleotide sequence by an RNA polymerase to form an RNA molecule. A TRE relies on one or more trans-acting molecules, such as transcription factors, to regulate transcription. Thus, one TRE may regulate transcription in different ways when it is in contact with different trans-acting molecules, for example, when it is in different types of cells. A TRE may comprise one or more promoter elements and/or enhancer elements. A skilled artisan would appreciate that the promoter and enhancer elements in a gene may be close in location, and the term "promoter" may refer to a sequence comprising a promoter element and an enhancer element. Thus, the term "promoter" does not exclude an enhancer element in the sequence. The promoter and enhancer elements do not need to be derived from the same gene or species, and the sequence of each promoter or enhancer element may be either identical or substantially identical to the corresponding endogenous sequence in the genome.

**[00126]** As used herein, the term "operably linked" is used to describe the connection between a TRE and a coding sequence to be transcribed. Typically, gene expression is placed under the control of a TRE comprising one or more promoter and/or enhancer elements. The coding sequence is "operably linked" to the TRE if the transcription of the coding sequence is controlled or influenced by the TRE. The promoter and enhancer elements of the TRE may be in any orientation and/or distance from the coding sequence, as long as the desired transcriptional activity is obtained. In certain embodiments, the TRE is upstream from the coding sequence.

**[00127]** In the instant disclosure, nucleotide positions in an antibody coding sequence (e.g., an antibody heavy chain coding sequence or an antibody light chain coding sequence) are specified relative to the first nucleotide of the start codon. The first nucleotide of a start codon is position 1; the nucleotides 5′ to the first nucleotide of the start codon have negative numbers; the nucleotides 3′ to the first nucleotide of the start codon have positive numbers.

**[00128]** As used herein, the term "expression cassette" refers to a polynucleotide sequence comprising, from 5′ to 3′, a transcriptional regulatory element (TRE), a coding sequence encoding a polypeptide, and a polyadenylation sequence. In certain embodiments, an intron is present between the TRE and the coding sequence. In certain embodiments, the coding sequence encodes an antibody heavy chain or an antibody light chain.

**[00129]** As used herein, the term "effective amount" in the context of the administration of an AAV to a subject refers to the amount of the AAV that achieves a desired prophylactic or therapeutic effect.

**[00130]** As used herein, the term "about" or "approximately" when referring to a

measurable value, such as the expression level of an antibody (e.g., an antibody heavy chain and antibody light chain), encompasses variations of ±20% or ±10%, ±5%, ±1%, or ±0.1% of a given value or range, as are appropriate to perform the methods disclosed herein.

## II. Adeno-Associated Virus Compositions

**[00131]** In one aspect, provided herein are novel rAAV genomes comprising a transcriptional regulatory element (TRE) operably linked to at least a portion of an antibody coding sequence (e.g., an anti-C5 antibody heavy chain coding sequence and/or anti-C5 antibody light chain coding sequence). The rAAV genomes provided herein are useful for extrachromosomal expression of an antibody in a cell comprising the rAAV genome.

**[00132]** The rAAV genome can be used to express antibodies in any mammalian cells (*e.g.*, human cells). Thus, the TRE can be active in any mammalian cells (*e.g.*, human cells). In certain embodiments, the TRE is active in a broad range of human cells. Such TREs may comprise constitutive promoter and/or enhancer elements including cytomegalovirus (CMV) promoter/enhancer (*e.g.*, comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 54, 55, or 56), SV40 promoter, chicken ACTB promoter (*e.g.*, comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 47 or 57), JeT promoter (*e.g.*, comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 58), smCBA promoter (*e.g.*, comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 59), human elongation factor 1 alpha (EF1α) promoter (*e.g.*, comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 39), minute virus of mouse (MVM) intron which comprises transcription factor binding sites (*e.g.*, comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 30 or 61), human phosphoglycerate kinase (PGK1) promoter, human ubiquitin C (Ubc) promoter, human beta actin promoter, human neuron-specific enolase (ENO2) promoter, human beta-glucuronidase (GUSB) promoter, a rabbit beta-globin element (*e.g.*, comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 41), human calmodulin 1 (CALM1) promoter (*e.g.*, comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 44), and/or human Methyl-CpG Binding Protein 2 (MeCP2) promoter. Any of these TREs can be combined in any order to drive efficient transcription. For example, an rAAV genome may comprise a CMV enhancer, a CBA promoter, and the splice acceptor from exon 3 of the rabbit beta-globin gene, collectively called a CAG promoter (*e.g.*, comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 42). For example, an rAAV genome may comprise a hybrid of CMV enhancer and CBA promoter followed by a splice donor and splice acceptor, collectively called a CASI promoter region (*e.g.*, comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 48 or 65).

**[00133]** Alternatively, the TRE may be a tissue-specific TRE, *i.e.*, it is active in specific tissue(s) and/or organ(s). A tissue-specific TRE comprises one or more tissue-specific promoter and/or enhancer elements, and optionally one or more constitutive promoter and/or enhancer elements. A skilled artisan would appreciate that tissue-specific promoter and/or enhancer elements can be isolated from genes specifically expressed in the tissue by methods well known in the art.

**[00134]** In certain embodiments, the TRE is liver-specific (e.g., hepatocyte-specific). Exemplary liver-specific TREs may comprise one or more elements selected from the group consisting of human albumin promoter, human transthyretin (TTR) promoter (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 66), human APOE/C-I hepatic control region (HCR) 1 (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 25 or 68), human APOH promoter, and human SERPINA1 (hAAT) promoter (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 26, 69 or 70) or a hepatic specific regulatory module thereof (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 71). In certain embodiments, an hAAT promoter

region comprises a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 72. In certain embodiments, the liver-specific TRE comprises the TBG SERPINA7 promoter as described in Yan et al. (Gene (2016) 506, 289-294) (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 116). In certain embodiments, the liver-specific TRE comprises the TBG SERPINA7 promoter as described in Hayashi et al. (Molecular Endocrinology (1993) 7(8), 1049-1060) (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 117). In certain embodiments, the liver-specific TRE comprises the hAAT SERPINA1 promoter as described in Hafenrichter et al. (Blood (1994) 84(10), 3394-3404) (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 118). In certain embodiments, the liver-specific TRE comprises the TTR promoter as described in Costa et al. (Molecular and Cellular Biology (1988) 8(1), 81-90) (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 119). In certain embodiments, the liver-specific TRE comprises the ApoA2 promoter as described in Kan et al. (Nucleic Acids Research (1999) 27(4), 1104-1117) (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 120). In certain embodiments, the liver-specific TRE comprises the albumin promoter as described in Tang et al. (Biomedical Reports (2017) 6, 627-632) (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 121). In certain embodiments, the liver-specific TRE comprises the modified fibrinogen promoter as described in Kyostio-Moore et al. (Molecular Therapy (2016) 3, 16006) (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 122). In certain embodiments, the liver-specific TRE comprises the minimum human APOE/C-I hepatic control region (HCR) 1 promoter as described in Dang et al. (J. Biol. Chem. (1995) 270(38), 22557-85) (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 123).

In certain embodiments, the liver-specific TRE comprises the human APOE/C-I hepatic control region (HCR) 2 promoter as described in Allan et al. (J. Biol. Chem. (1995) 270(44), 26278-81) (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 124). More liver-specific promoter elements are disclosed in WO 2009/130208 and Kramer et al. (Molecular Therapy (2003) 7, 375–385), which are incorporated by reference herein in their entirety.

**[00135]** In certain embodiments, the rAAV genome comprises two or more TREs, optionally comprising at least one of the TREs disclosed above. A skilled person in the art would appreciate that any of these TREs can be combined in any order, and combinations of a constitutive TRE and a tissue-specific TRE can drive efficient and tissue-specific transcription. For example, in certain embodiments, the rAAV genome comprises a human HCR1 (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 25, 68, or 123) and a human EF-1α promoter (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 39), optionally wherein the human HCR1 is 5′ to the human EF-1α promoter. In certain embodiments, the rAAV genome comprises a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence nucleotide set forth in SEQ ID NO: 60.

**[00136]** Similarly, combinations of two or more tissue-specific TREs can drive efficient and tissue-specific transcription. For example, in certain embodiments, the rAAV genome comprises a human HCR1 (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 25, 68, or 123) and a hAAT promoter (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 26), optionally wherein the human HCR1 is 5′ to the hAAT promoter. In certain embodiments, the rAAV genome comprises a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence set forth in SEQ ID NO: 27. In certain embodiments, the rAAV genome comprises a human HCR1 (e.g., comprising the nucleotide sequence set forth in SEQ ID NO: 25) and a hAAT promoter (e.g., comprising the nucleotide sequence set forth

in SEQ ID NO: 26), optionally wherein the human HCR1 is 5′ to the hAAT promoter. In certain embodiments, the rAAV genome comprises the nucleotide sequence set forth in SEQ ID NO: 27.

**[00137]** In certain embodiments, the rAAV genome comprises a hepatic specific regulatory module of hAAT promoter (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 71) and a human TTR promoter (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 66), optionally wherein the hepatic specific regulatory module is 5′ to the human TTR promoter. In certain embodiments, the rAAV genome comprises a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence set forth in SEQ ID NO: 67. In certain embodiments, the rAAV genome comprises a hepatic specific regulatory module of hAAT promoter (e.g., comprising the nucleotide sequence set forth in SEQ ID NO: 71) and a human TTR promoter (e.g., comprising the nucleotide sequence set forth in SEQ ID NO: 66), optionally wherein the hepatic specific regulatory module is 5′ to the human TTR promoter. In certain embodiments, the rAAV genome comprises the nucleotide sequence set forth in SEQ ID NO: 67.

**[00138]** In certain embodiments, the rAAV genome further comprises an intron element 5′ to the at least a portion of an antibody coding sequence. Such intron elements can increase transgene expression, for example, by reducing transcriptional silencing and enhancing mRNA export from the nucleus to the cytoplasm. In certain embodiments, the rAAV genome comprises from 5′ to 3′: a TRE, an intron element, and the at least a portion of an antibody coding sequence.

**[00139]** The intron element can comprise at least a portion of a native intron sequence of an immunoglobulin gene, or the intron element can be an exogenous intron element (*e.g.*, comprising at least an intron sequence from a different species or a different gene from the same species, and/or a synthetic intron sequence). In certain embodiments, the intron element is an exogenous intron element comprising at least a portion of an intron sequence from a different species. In certain embodiments, the intron element is an exogenous intron element comprising at least a portion of an intron sequence from a different gene from the same species. In certain embodiments, the intron element is an exogenous intron element comprising a synthetic intron sequence. In certain embodiments, the intron element is an exogenous intron

element comprising a combination of at least an intron sequence from a different species or a different gene from the same species, and/or a synthetic intron sequence.

**[00140]** A skilled worker will appreciate that intron elements can be designed to mediate RNA splicing by introducing any consensus splicing motifs known in the art (e.g., in Sibley et al., (2016) Nature Reviews Genetics, 17, 407-21, which is incorporated by reference herein in its entirety). Exemplary intron sequences are provided in Lu et al. (2013) Molecular Therapy 21(5): 954-63, and Lu et al. (2017) Hum. Gene Ther. 28(1): 125-34, which are incorporated by reference herein in their entirety.

**[00141]** In certain embodiments, the rAAV genome comprises an exogenous intron element. In certain embodiments, the rAAV comprises an SV40 intron element (*e.g.*, comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 29), a minute virus of mouse (MVM) intron (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 30 or 61), or a synthetic intron (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 45). In certain embodiments, the rAAV genome comprises an SV40 intron element (e.g., comprising the nucleotide sequence set forth in SEQ ID NO: 29), a minute virus of mouse (MVM) intron element (e.g., comprising the nucleotide sequence set forth in SEQ ID NOs: 30 and 61), or a synthetic intron (e.g., comprising a nucleotide sequence set forth in SEQ ID NO: 45).

**[00142]** In certain embodiments, the rAAV genome comprises from 5′ to 3′: a TRE and an intron element. In certain embodiments, the combined TRE and intron element has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 43 or 50. In certain embodiments, the combined TRE and intron element comprises a nucleotide sequence of SEQ ID NO: 43 or 50. In certain embodiments, the combined TRE and intron element consists of a nucleotide sequence of SEQ ID NO: 43 or 50.

**[00143]** In certain embodiments, the rAAV genome disclosed herein further comprises a transcription terminator (e.g., a polyadenylation sequence). In certain embodiments, the transcription terminator is 3′ to the at least a portion of an antibody coding sequence. The transcription terminator may be any sequence that effectively terminates transcription, and a skilled artisan would appreciate that such sequences can be isolated from any genes that are

expressed in the cell in which transcription of the at least a portion of an antibody coding sequence is desired. In certain embodiments, the transcription terminator comprises a polyadenylation sequence. In certain embodiments, the polyadenylation sequence is identical or substantially identical to the endogenous polyadenylation sequence of an immunoglobulin gene. In certain embodiments, the polyadenylation sequence is an exogenous polyadenylation sequence. In certain embodiments, the polyadenylation sequence is an SV40 polyadenylation sequence (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 31, 34, or 35, or a nucleotide sequence complementary thereto). In certain embodiments, the polyadenylation sequence comprises the nucleotide sequence set forth in SEQ ID NO: 31. In certain embodiments, the polyadenylation sequence consists of the nucleotide sequence set forth in SEQ ID NO: 31. In certain embodiments, the polyadenylation sequence is a bovine growth hormone (BGH) polyadenylation sequence (e.g., comprising a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33, or a nucleotide sequence complementary thereto). In certain embodiments, the polyadenylation sequence comprises the nucleotide sequence set forth in SEQ ID NO: 32. In certain embodiments, the polyadenylation sequence consists of the nucleotide sequence set forth in SEQ ID NO: 32.

**[00144]** In certain embodiments, the rAAV genome comprises from 5′ to 3′: a TRE, an intron element, at least a portion of an antibody coding sequence, and a polyadenylation sequence. In certain embodiments, the TRE has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 25-27, 36, 39, 42, 44, 46-49, 54-60, or 65-72; the intron element has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 29, 30, or 61; the at least a portion of an antibody coding sequence has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 52, 53, 62, 63, 83, 94, 95, 97, 98, 100, 101, 103, 104, 106, 107, 109, 110, 112, 113, 114, 115, 131, and 132; and/or the polyadenylation sequence has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 31, 33, 34, or 35.

**[00145]** In certain embodiments, the TRE comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 25-27, 36, 39, 42, 44, 46-49, 54-60, and 65-72; the intron element comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 29, 30, and 61; the at least a portion of an antibody coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 52; and/or the polyadenylation sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 31, 33, 34, and 35.

**[00146]** In certain embodiments, the TRE comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 25-27, 36, 39, 42, 44, 46-49, 54-60, and 65-72; the intron element comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 29, 30, and 61; the at least a portion of an antibody coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 53; and/or the polyadenylation sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 31, 33, 34, and 35.

**[00147]** In certain embodiments, the TRE comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 25-27, 36, 39, 42, 44, 46-49, 54-60, and 65-72; the intron element comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 29, 30, and 61; the at least a portion of an antibody coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 62; and/or the polyadenylation sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 31, 33, 34, and 35.

**[00148]** In certain embodiments, the TRE comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 25-27, 36, 39, 42, 44, 46-49, 54-60, and 65-72; the intron element comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 29, 30, and 61; the at least a portion of an antibody coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 63; and/or the polyadenylation sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 31, 33, 34, and 35.

**[00149]** In certain embodiments, the TRE comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 25-27, 36, 39, 42, 44, 46-49, 54-60, and 65-72; the intron element comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 29, 30, and 61; the at least a portion of an antibody coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 83; and/or the polyadenylation sequence

comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 31, 33, 34, and 35.

**[00150]** In certain embodiments, the TRE comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 25, 26, or 27; the intron element comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 29; the at least a portion of an antibody coding sequence comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 52; and/or the polyadenylation sequence comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 33.

**[00151]** In certain embodiments, the TRE comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 25, 26, or 27; the intron element comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 29; the at least a portion of an antibody coding sequence comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 62; and/or the polyadenylation sequence comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 33.

**[00152]** In certain embodiments, the TRE comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 25, 26, or 27; the intron element comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 29; the at least a portion of an antibody coding sequence comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 83; and/or the polyadenylation sequence comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 33.

**[00153]** In certain embodiments, the TRE comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 66, 67, or 71; the intron element comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 30 or 61; the at least a portion of an antibody coding sequence comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 53; and/or the polyadenylation sequence comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 31.

**[00154]** In certain embodiments, the TRE comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 66, 67, or 71; the intron element comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 30 or 61; the at least a portion of an antibody coding sequence comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 63; and/or the polyadenylation sequence comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 31.

**[00155]** In certain embodiments, the rAAV genome comprises a nucleotide sequence at least 80% (e.g., at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5%) identical to any one of SEQ ID NO: 88, 89, 90, or 91. In certain embodiments, the rAAV genome comprises the nucleotide sequence set forth in any one of SEQ ID NO: 88, 89, 90, or 91. In certain embodiments, the rAAV genome consists of the nucleotide sequence set forth in any one of SEQ ID NO: 88, 89, 90, or 91.

**[00156]** In certain embodiments, the rAAV genome comprises a nucleotide sequence at least 80% (e.g., at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91% 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5%) identical to SEQ ID NO: 88. In certain embodiments, the rAAV genome comprises the nucleotide sequence set forth in SEQ ID NO: 88. In certain embodiments, the rAAV genome consists of the nucleotide sequence set forth in SEQ ID NO: 88.

**[00157]** In certain embodiments, the rAAV genome comprises a nucleotide sequence at least 80% (e.g., at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91% 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5%) identical to SEQ ID NO: 89. In certain embodiments, the rAAV genome comprises the nucleotide sequence set forth in SEQ ID NO: 89. In certain embodiments, the rAAV genome consists of the nucleotide sequence set forth in SEQ ID NO: 89.

**[00158]** In certain embodiments, the rAAV genome comprises a nucleotide sequence at least 80% (e.g., at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91% 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5%) identical to SEQ ID NO: 90. In certain embodiments, the rAAV genome comprises the nucleotide sequence set forth in SEQ ID NO: 90. In certain embodiments, the rAAV genome consists of the nucleotide sequence set forth in SEQ ID NO: 90.

**[00159]** In certain embodiments, the rAAV genome comprises a nucleotide sequence at least 80% (e.g., at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91% 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5%) identical to SEQ ID NO: 91. In certain embodiments, the rAAV genome comprises the nucleotide sequence set forth in SEQ ID NO: 91. In certain embodiments, the rAAV genome consists of the nucleotide sequence set forth in SEQ ID NO: 91.

**[00160]** In certain embodiments, the rAAV genomes disclosed herein further comprise a 5′ inverted terminal repeat (5′ ITR) nucleotide sequence 5′ to the TRE, and a 3′ inverted terminal repeat (3′ ITR) nucleotide sequence 3′ to the polyadenylation sequence associated with an antibody light chain coding sequence. ITR sequences from any AAV serotype or variant thereof can be used in the rAAV genomes disclosed herein. The 5′ and 3′ ITR can be from an

AAV of the same serotype or from AAVs of different serotypes. Exemplary ITRs for use in the rAAV genomes disclosed herein are set forth in SEQ ID NOs: 14, 18, 19, 20, 21, and 32, herein.

**[00161]** In certain embodiments, the 5′ ITR or 3′ ITR is from AAV2. In certain embodiments, both the 5′ ITR and the 3′ ITR are from AAV2. In certain embodiments, the 5′ ITR nucleotide sequence has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 14, or the 3′ ITR nucleotide sequence has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 18. In certain embodiments, the 5′ ITR nucleotide sequence has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 14, and the 3′ ITR nucleotide sequence has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 18. In certain embodiments, the rAAV genome comprises a nucleotide sequence set forth in SEQ ID NO: 43, a 5′ ITR nucleotide sequence having the sequence of SEQ ID NO: 14, and a 3′ ITR nucleotide sequence having the sequence of SEQ ID NO: 18.

**[00162]** In certain embodiments, the 5′ ITR or 3′ ITR are from AAV5. In certain embodiments, both the 5′ ITR and 3′ ITR are from AAV5. In certain embodiments, the 5′ ITR nucleotide sequence has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 20, or the 3′ ITR nucleotide sequence has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 21. In certain embodiments, the 5′ ITR nucleotide sequence has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 20, and the 3′ ITR nucleotide sequence has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 21. In certain embodiments, the rAAV genome comprises a nucleotide sequence set forth in any one of SEQ ID NO: 43, a 5′ ITR nucleotide sequence having the sequence of SEQ ID NO: 20, and a 3′ ITR nucleotide sequence having the sequence of SEQ ID NO: 21.

**[00163]** In certain embodiments, the 5′ ITR nucleotide sequence and the 3′ ITR nucleotide sequence are substantially complementary to each other (e.g., are complementary to each other except for mismatch at 1, 2, 3, 4, or 5 nucleotide positions in the 5′ or 3′ ITR).

**[00164]** In certain embodiments, the 5′ ITR or the 3′ ITR is modified to reduce or abolish resolution by Rep protein ("non-resolvable ITR"). In certain embodiments, the non-resolvable ITR comprises an insertion, deletion, or substitution in the nucleotide sequence of the terminal resolution site. Such modification allows formation of a self-complementary, double-stranded DNA genome of the AAV after the rAAV genome is replicated in an infected cell. Exemplary non-resolvable ITR sequences are known in the art (see e.g., those provided in U.S. Patent Nos. 7,790,154 and 9,783,824, which are incorporated by reference herein in their entirety). In certain embodiments, the 5′ ITR comprises a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 19. In certain embodiments, the 5′ ITR consists of a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 19. In certain embodiments, the 5′ ITR consists of the nucleotide sequence set forth in SEQ ID NO: 19. In certain embodiments, the 3′ ITR comprises a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 32. In certain embodiments, the 5′ ITR consists of a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 32. In certain embodiments, the 3′ ITR consists of the nucleotide sequence set forth in SEQ ID NO: 32. In certain embodiments, the 5′ ITR consists of the nucleotide sequence set forth in SEQ ID NO: 19, and the 3′ ITR consists of the nucleotide sequence set forth in SEQ ID NO: 32. In certain embodiments, the 5′ ITR consists of the nucleotide sequence set forth in SEQ ID NO: 19, and the 3′ ITR consists of the nucleotide sequence set forth in SEQ ID NO: 32.

**[00165]** In certain embodiments, the 5′ ITR is flanked by an additional nucleotide sequence derived from a wild-type AAV2 genomic sequence. In certain embodiments, the 5′ ITR is flanked by an additional 46 bp sequence derived from a wild-type AAV2 sequence that is adjacent to a wild-type AAV2 ITR in an AAV2 genome. In certain embodiments, the additional 46 bp sequence is 3′ to the 5′ ITR in the rAAV genome. In certain embodiments, the 46 bp sequence consists of the nucleotide sequence set forth in SEQ ID NO: 74.

**[00166]** In certain embodiments, the 3′ ITR is flanked by an additional nucleotide sequence derived from a wild-type AAV2 genomic sequence. In certain embodiments, the 3′ ITR is flanked by an additional 37 bp sequence derived from a wild-type AAV2 sequence that is adjacent to a wild-type AAV2 ITR in an AAV2 genome. *See, e.g.,* Savy et al., Human Gene Therapy Methods (2017) 28(5): 277-289 (which is hereby incorporated by reference herein in

its entirety). In certain embodiments, the additional 37 bp sequence is 5′ to the 3′ ITR in the rAAV genome. In certain embodiments, the 37 bp sequence consists of the nucleotide sequence set forth in SEQ ID NO: 73.

**[00167]** In another aspect, provided herein is a polynucleotide comprising a nucleic acid sequence that is at least 80% (e.g., at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91% 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to the nucleic acid sequence set forth in SEQ ID NO: 84, 85, 86, or 87. In certain embodiments, the polynucleotide comprises or consists of the nucleic acid sequence set forth in SEQ ID NO: 84, 85, 86, or 87.

**[00168]** In another aspect, provided herein are novel rAAV compositions comprising an AAV capsid comprising an AAV capsid protein, an rAAV genome as disclosed herein (e.g., an rAAV genome comprising a transcriptional regulatory element operably linked to an antibody coding sequence (*e.g.*, an antibody heavy chain or light chain coding sequence), allowing for extrachromosomal expression of an antibody in a cell transduced with the AAV).

**[00169]** A capsid protein from any AAV capsid known the art can be used in the rAAV compositions disclosed herein, including, without limitation, a capsid protein from an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, or AAV9 serotype. For example, in certain embodiments, the capsid protein comprises an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of amino acids 203-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17. In certain embodiments, the capsid protein comprises an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of amino acids 203-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17, wherein: the amino acid in the capsid protein corresponding to amino acid 206 of SEQ ID NO: 16 is C; the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H; the amino acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q; the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A; the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N; the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S; the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I; the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 590 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G or Y; the amino acid in the capsid

protein corresponding to amino acid 681 of SEQ ID NO: 16 is M; the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K; the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C; or, the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G, and the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H, the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A, and the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C. In certain embodiments, the capsid protein comprises the amino acid sequence of amino acids 203-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

**[00170]** For example, in certain embodiments, the capsid protein comprises an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of amino acids 138-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17. In certain embodiments, the capsid protein comprises an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of amino acids 138-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17, wherein: the amino acid in the capsid protein corresponding to amino acid 151 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 160 of SEQ ID NO: 16 is D; the amino acid in the capsid protein corresponding to amino acid 206 of SEQ ID NO: 16 is C; the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H; the amino

acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q; the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A; the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N; the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S; the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I; the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 590 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G or Y; the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M; the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K; the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C; or, the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G, and the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H, the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A, and the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C. In certain embodiments, the capsid protein comprises the amino acid sequence of amino acids 138-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

**[00171]** For example, in certain embodiments, the capsid protein comprises an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of amino acids 1-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or

17. In certain embodiments, the capsid protein comprises an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of amino acids 1-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17, wherein: the amino acid in the capsid protein corresponding to amino acid 2 of SEQ ID NO: 16 is T; the amino acid in the capsid protein corresponding to amino acid 65 of SEQ ID NO: 16 is I; the amino acid in the capsid protein corresponding to amino acid 68 of SEQ ID NO: 16 is V; the amino acid in the capsid protein corresponding to amino acid 77 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 119 of SEQ ID NO: 16 is L; the amino acid in the capsid protein corresponding to amino acid 151 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 160 of SEQ ID NO: 16 is D; the amino acid in the capsid protein corresponding to amino acid 206 of SEQ ID NO: 16 is C; the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H; the amino acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q; the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A; the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N; the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S; the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I; the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 590 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G or Y; the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M; the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K; the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C; or, the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 2 of SEQ ID NO: 16 is T, and the amino acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 65 of SEQ ID NO: 16 is I, and the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is Y. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 77 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 119 of

SEQ ID NO: 16 is L, and the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G, and the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H, the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A, and the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R. In certain embodiments, the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C. In certain embodiments, the capsid protein comprises the amino acid sequence of amino acids 1-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

**[00172]** In certain embodiments, the AAV capsid comprises two or more of: (a) a capsid protein comprising the amino acid sequence of amino acids 203-736 of SEQ ID NO: 1, 2, 3, 4, 6, 7, 10, 11, 12, 13, 15, 16, or 17; (b) a capsid protein comprising the amino acid sequence of amino acids 138-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 15, 16, or 17; and (c) a capsid protein comprising the amino acid sequence of amino acids 1-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17. In certain embodiments, the AAV capsid comprises: (a) a capsid protein having an amino acid sequence consisting of amino acids 203-736 of SEQ ID NO: 1, 2, 3, 4, 6, 7, 10, 11, 12, 13, 15, 16, or 17; (b) a capsid protein having an amino acid sequence consisting of amino acids 138-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 15, 16, or 17; and (c) a capsid protein having an amino acid sequence consisting of amino acids 1-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

**[00173]** In certain embodiments, the AAV capsid comprises one or more of: (a) a capsid protein comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the sequence of amino acids 203-736 of SEQ ID NO: 8; (b) a capsid protein comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the sequence of amino acids 138-736 of SEQ ID NO: 8; and (c) a capsid protein comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the sequence of amino acids 1-736 of SEQ ID NO: 8. In certain embodiments, the AAV capsid comprises one or more of: (a) a capsid protein comprising the amino acid sequence of amino acids 203-736 of SEQ ID NO: 8; (b) a capsid protein comprising the amino acid sequence of amino acids 138-736 of SEQ ID NO: 8; and (c) a capsid protein comprising the amino acid sequence of amino acids 1-736 of SEQ ID NO: 8. In certain embodiments, the AAV capsid comprises two or more of: (a) a capsid protein comprising the amino acid sequence of amino acids 203-736 of SEQ ID NO: 8; (b) a capsid protein comprising the amino acid sequence of amino acids 138-736 of SEQ ID NO: 8; and (c) a capsid protein comprising the amino acid sequence of amino acids 1-736 of SEQ ID NO: 8. In certain embodiments, the AAV capsid comprises: (a) a capsid protein having an amino acid sequence consisting of amino acids 203-736 of SEQ ID NO: 8; (b) a capsid protein having an amino acid sequence consisting of amino acids 138-736 of SEQ ID NO: 8; and (c) a capsid protein having an amino acid sequence consisting of amino acids 1-736 of SEQ ID NO: 8.

**[00174]** In certain embodiments, the AAV capsid comprises one or more of: (a) a capsid protein comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the sequence of amino acids 203-736 of SEQ ID NO: 11; (b) a capsid protein comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the sequence of amino acids 138-736 of SEQ ID NO: 11; and (c) a capsid protein comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the sequence of amino acids 1-736 of SEQ ID NO: 11. In certain embodiments, the AAV capsid comprises one or more of: (a) a capsid protein comprising the amino acid sequence of amino acids 203-736 of SEQ ID NO: 11; (b) a capsid protein comprising the amino acid sequence of amino acids 138-736 of SEQ ID NO: 11; and (c) a capsid protein comprising the amino acid sequence of amino acids 1-736 of SEQ ID NO: 11. In certain embodiments, the AAV capsid comprises two or more of: (a) a capsid protein comprising the amino acid sequence of amino acids 203-736 of SEQ ID NO: 11; (b) a capsid protein comprising the amino acid sequence of amino acids 138-736 of SEQ ID NO: 11; and

(c) a capsid protein comprising the amino acid sequence of amino acids 1-736 of SEQ ID NO: 11. In certain embodiments, the AAV capsid comprises: (a) a capsid protein having an amino acid sequence consisting of amino acids 203-736 of SEQ ID NO: 11; (b) a capsid protein having an amino acid sequence consisting of amino acids 138-736 of SEQ ID NO: 11; and (c) a capsid protein having an amino acid sequence consisting of amino acids 1-736 of SEQ ID NO: 11.

**[00175]** In certain embodiments, the AAV capsid comprises one or more of: (a) a capsid protein comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the sequence of amino acids 203-736 of SEQ ID NO: 13; (b) a capsid protein comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the sequence of amino acids 138-736 of SEQ ID NO: 13; and (c) a capsid protein comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the sequence of amino acids 1-736 of SEQ ID NO: 13. In certain embodiments, the AAV capsid comprises one or more of: (a) a capsid protein comprising the amino acid sequence of amino acids 203-736 of SEQ ID NO: 13; (b) a capsid protein comprising the amino acid sequence of amino acids 138-736 of SEQ ID NO: 13; and (c) a capsid protein comprising the amino acid sequence of amino acids 1-736 of SEQ ID NO: 13. In certain embodiments, the AAV capsid comprises two or more of: (a) a capsid protein comprising the amino acid sequence of amino acids 203-736 of SEQ ID NO: 13; (b) a capsid protein comprising the amino acid sequence of amino acids 138-736 of SEQ ID NO: 13; and (c) a capsid protein comprising the amino acid sequence of amino acids 1-736 of SEQ ID NO: 13. In certain embodiments, the AAV capsid comprises: (a) a capsid protein having an amino acid sequence consisting of amino acids 203-736 of SEQ ID NO: 13; (b) a capsid protein having an amino acid sequence consisting of amino acids 138-736 of SEQ ID NO: 13; and (c) a capsid protein having an amino acid sequence consisting of amino acids 1-736 of SEQ ID NO: 13.

**[00176]** In certain embodiments, the AAV capsid comprises one or more of: (a) a capsid protein comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the sequence of amino acids 203-736 of SEQ ID NO: 16; (b) a capsid protein comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the sequence of amino acids 138-736 of SEQ ID NO: 16; and (c) a capsid protein

comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the sequence of amino acids 1-736 of SEQ ID NO: 16. In certain embodiments, the AAV capsid comprises one or more of: (a) a capsid protein comprising the amino acid sequence of amino acids 203-736 of SEQ ID NO: 16; (b) a capsid protein comprising the amino acid sequence of amino acids 138-736 of SEQ ID NO: 16; and (c) a capsid protein comprising the amino acid sequence of amino acids 1-736 of SEQ ID NO: 16. In certain embodiments, the AAV capsid comprises two or more of: (a) a capsid protein comprising the amino acid sequence of amino acids 203-736 of SEQ ID NO: 16; (b) a capsid protein comprising the amino acid sequence of amino acids 138-736 of SEQ ID NO: 16; and (c) a capsid protein comprising the amino acid sequence of amino acids 1-736 of SEQ ID NO: 16. In certain embodiments, the AAV capsid comprises: (a) a capsid protein having an amino acid sequence consisting of amino acids 203-736 of SEQ ID NO: 16; (b) a capsid protein having an amino acid sequence consisting of amino acids 138-736 of SEQ ID NO: 16; and (c) a capsid protein having an amino acid sequence consisting of amino acids 1-736 of SEQ ID NO: 16.

**[00177]** The rAAV genomes of the disclosure can be used to express any antibody heavy chain and antibody light chain known in the art. In one aspect, provided herein are rAAV genomes comprising a TRE operably linked to at least a portion of an antibody coding sequence (e.g., an antibody heavy chain coding sequence and/or an antibody light chain coding sequence). Non-limiting examples of antibodies include, anti-C5 antibodies (e.g., eculizumab, ravulizumab, and pozelimab), anti-Factor D antibodies (e.g., lampalizumab), anti-mannose-binding protein-associated serine protease 2 (MASP-2) antibodies (e.g., narsoplimab), anti-kallikrein antibodies (e.g., lanadelumab), anti-interleukin 1 beta antibodies (e.g., canakinumab), anti-interferon gamma antibodies (e.g., emapalumab), anti-PCSK9 antibodies (e.g., evolocumab and alirocumab), anti-coagulation factor IX and factor X antibodies (e.g., bispecific antibody emicizumab), and anti-VEGF antibodies (e.g., ranibizumab).

**[00178]** In another aspect, the instant disclosure provides pharmaceutical compositions comprising an AAV as disclosed herein together with a pharmaceutically acceptable excipient, adjuvant, diluent, vehicle or carrier, or a combination thereof. A "pharmaceutically acceptable carrier" includes any material which, when combined with an active ingredient of a composition, allows the ingredient to retain biological activity and without causing disruptive physiological reactions, such as an unintended immune reaction. Pharmaceutically acceptable carriers include water, phosphate buffered saline, emulsions such as oil/water emulsion, and wetting agents. Compositions comprising such carriers are formulated by well-known

conventional methods such as those set forth in Remington's Pharmaceutical Sciences, current Ed., Mack Publishing Co., Easton Pa. 18042, USA; A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy", 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H. C. Ansel et al, 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A. H. Kibbe et al, 3rd ed. Amer. Pharmaceutical Assoc.

**[00179]** In another aspect, the instant disclosure provides pharmaceutical compositions comprising an AAV as disclosed herein together with a pharmaceutically acceptable excipient, adjuvant, diluent, vehicle or carrier, or a combination thereof. A "pharmaceutically acceptable carrier" includes any material which, when combined with an active ingredient of a composition, allows the ingredient to retain biological activity and without causing disruptive physiological reactions, such as an unintended immune reaction. Pharmaceutically acceptable carriers include water, phosphate buffered saline, emulsions such as oil/water emulsion, and wetting agents. Compositions comprising such carriers are formulated by well-known conventional methods such as those set forth in Remington's Pharmaceutical Sciences, current Ed., Mack Publishing Co., Easton Pa. 18042, USA; A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy", 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H. C. Ansel et al, 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A. H. Kibbe et al, 3rd ed. Amer. Pharmaceutical Assoc.

## III. Method of Use

**[00180]** In another aspect, the instant disclosure provides methods for expressing an antibody in a cell (e.g., an antibody heavy chain and light chain). The methods generally comprise transducing the cell with an rAAV as disclosed herein. Such methods lead to high-level expression and secretion of antibodies. Accordingly, in certain embodiments, the methods disclosed herein involve transducing the cell with an rAAV as disclosed herein.

**[00181]** The methods disclosed herein can be applied to any cell (e.g., liver cells) in which expression of an antibody is desired. Accordingly, in certain embodiments, the method is applied to cells in the liver. In certain embodiments, the method is applied to hepatocytes.

**[00182]** The methods disclosed herein can be performed *in vitro* for research purposes or can be performed *ex vivo* or *in vivo* for therapeutic purposes.

**[00183]** In certain embodiments, the cell to be transduced is in a mammalian subject and the AAV is administered to the subject in an amount effective to transduce the cell in the

subject. Accordingly, in certain embodiments, the instant disclosure provides a method for treating a subject having a disease or disorder that would benefit from the expression and secretion of an antibody that specifically binds a therapeutic target, the method generally comprising administering to the subject an effective amount of an rAAV as disclosed herein. In certain embodiments, the antibody specifically binds complement C5 and the disease or disorder is associated with complement C5 activity. The subject can be a human subject or a rodent subject (e.g., a mouse) containing human liver cells. Suitable mouse subjects include without limitation, mice into which human liver cells (e.g., human hepatocytes) have been engrafted. Any disease or disorder associated with complement C5 activity can be treated using the methods disclosed herein. Suitable diseases or disorders include, without limitation, paroxysmal nocturnal hemoglobinuria (PNH), neuromyelitis optica spectrum disorder (NMOSD), atypical hemolytic uremic syndrome (aHUS), myasthenia gravis, hematopoietic stem cell transplantation-transplant-associated thrombotic microangiopathy (HSCT-TMA), complement-mediated thrombotic microangiopathy (CM-TMA), Guillain-Barré syndrome, amyotrophic lateral sclerosis (ALS), primary progressive multiple sclerosis (PPMS), multifocal motor neuropathy, antibody-mediated kidney rejection, C3 glomerulopathy, age-related macular degeneration (AMD), AQP4 IgG-positive neuromyelitis optica, systemic lupus erythematosus, psoriasis, rheumatoid arthritis (RA), dermatomyositis, idiopathic membranous glomerulopathy, demyelinating neuropathy, complement hyperactivation, angiopathic thrombosis, protein losing enteropathy (CHAPLE) syndrome, geographic atrophy (GA), asthma, proliferative nephritis, and sepsis.

**[00184]** In certain embodiments, the instant disclosure provides a method for treating a subject having a disease or disorder associated with complement C5 activity, the method generally comprising administering to the subject an effective amount of an rAAV as disclosed herein. The subject can be a human subject, a non-human primate subject (*e.g.*, a cynomolgus), or a rodent subject (*e.g.*, a mouse) with aberrant complement C5 activity. Any disease or disorder associated with complement C5 activity can be treated using the methods disclosed herein. Suitable diseases or disorders include, without limitation, PNH, NMOSD, aHUS, myasthenia gravis, HSCT-TMA, CM-TMA, Guillain-Barré syndrome, ALS, PPMS, multifocal motor neuropathy, antibody-mediated kidney rejection, C3 glomerulopathy, AMD, AQP4 IgG-positive neuromyelitis optica, systemic lupus erythematosus, psoriasis, RA, dermatomyositis, idiopathic membranous glomerulopathy, demyelinating neuropathy, CHAPLE syndrome, geographic atrophy (GA), asthma, proliferative nephritis, and sepsis.

**[00185]** In certain embodiments, the foregoing methods employ an rAAV comprising an AAV capsid protein comprising the amino acid sequence of amino acids 203-736 of SEQ ID NO: 16, and an rAAV genome comprising 5′ to 3′ following genetic elements: a 5′ ITR (*e.g.*, the 5′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 14), a transcriptional regulatory element (*e.g.*, a TRE comprising the nucleotide sequence set forth in SEQ ID NO: 60), at least a portion of an antibody heavy chain coding sequence (*e.g.*, the antibody heavy chain coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 52, 62, or 83), a T2A peptide cleavage sequence (*e.g.*, the T2A peptide cleavage sequence of SEQ ID NO: 28), at least a portion of an antibody light chain coding sequence (*e.g.*, the antibody light chain coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 53 or 63), a polyadenylation sequence (*e.g.*, the SV40 polyadenylation sequence of SEQ ID NO: 31), and a 3′ ITR (*e.g.*, the 3′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 18).

**[00186]** In certain embodiments, the foregoing methods employ an rAAV comprising an AAV capsid protein comprising the amino acid sequence of amino acids 138-736 of SEQ ID NO: 16, and an rAAV genome comprising 5′ to 3′ following genetic elements: a 5′ ITR (*e.g.*, the 5′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 14), a transcriptional regulatory element (*e.g.*, a TRE comprising the nucleotide sequence set forth in SEQ ID NO: 27), an intron element (*e.g.*, the intron element comprising the nucleotide sequence set forth in SEQ ID NO: 29), at least a portion of an antibody heavy chain coding sequence (*e.g.*, the antibody heavy chain coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 52, 62, or 83), a T2A peptide cleavage sequence (*e.g.*, the T2A peptide cleavage sequence of SEQ ID NO: 28), at least a portion of an antibody light chain coding sequence (*e.g.*, the antibody light chain coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 53 or 63), a polyadenylation sequence (*e.g.*, the SV40 polyadenylation sequence of SEQ ID NO: 31), and a 3′ ITR (*e.g.*, the 3′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 18).

**[00187]** In certain embodiments, the foregoing methods employ an rAAV comprising an AAV capsid protein comprising the amino acid sequence of SEQ ID NO: 16, and an rAAV genome comprising 5′ to 3′ following genetic elements: a 5′ ITR (*e.g.*, the 5′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 14), a transcriptional regulatory element (*e.g.*, a TRE comprising the nucleotide sequence set forth in SEQ ID NO: 27), an intron element (*e.g.*, the intron element comprising the nucleotide sequence set forth in SEQ ID NO: 29), at least a portion of an antibody heavy chain coding sequence (*e.g.*, the antibody heavy chain

coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 52, 62, or 83), a polyadenylation sequence (*e.g.*, the BGH polyadenylation sequence of SEQ ID NO: 33), a transcriptional regulatory element (*e.g.*, a TRE comprising the nucleotide sequence set forth in SEQ ID NO: 67), an intron element (*e.g.*, the intron element comprising the nucleotide sequence set forth in SEQ ID NO: 30 or 61), at least a portion of an antibody light chain coding sequence (*e.g.*, the antibody light chain coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 53 or 63), a polyadenylation sequence (*e.g.*, the SV40 polyadenylation sequence of SEQ ID NO: 31), and a 3′ ITR (*e.g.*, the 3′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 18).

**[00188]** In certain embodiments, the foregoing methods employ an rAAV comprising an AAV capsid protein comprising the amino acid sequence of SEQ ID NO: 16, and an rAAV genome comprising 5′ to 3′ following genetic elements: a 5′ ITR (*e.g.*, the 5′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 14), a polyadenylation sequence (*e.g.*, the BGH polyadenylation sequence of SEQ ID NO: 33), at least a portion of an antibody heavy chain coding sequence (*e.g.*, the antibody heavy chain coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 52, 62, or 83), an intron element (*e.g.*, the intron element comprising the nucleotide sequence set forth in SEQ ID NO: 29), a transcriptional regulatory element (*e.g.*, a TRE comprising the nucleotide sequence set forth in SEQ ID NO: 27), a stuffer sequence (*e.g.*, a stuffer comprising the nucleotide sequence set forth in SEQ ID NO: 51), a transcriptional regulatory element (*e.g.*, a TRE comprising the nucleotide sequence set forth in SEQ ID NO: 67), an intron element (*e.g.*, the intron element comprising the nucleotide sequence set forth in SEQ ID NO: 30 or 61), at least a portion of an antibody light chain coding sequence (*e.g.*, the antibody light chain coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 53 or 63), a polyadenylation sequence (*e.g.*, the SV40 polyadenylation sequence of SEQ ID NO: 31), and a 3′ ITR (*e.g.*, the 3′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 18).

**[00189]** In certain embodiments, the foregoing methods employ an rAAV comprising an AAV capsid protein comprising the amino acid sequence of amino acids 203-736 of SEQ ID NO: 13, and an rAAV genome comprising 5′ to 3′ following genetic elements: a 5′ ITR (*e.g.*, the 5′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 14), a transcriptional regulatory element (*e.g.*, a TRE comprising the nucleotide sequence set forth in SEQ ID NO: 60), at least a portion of an antibody heavy chain coding sequence (*e.g.*, the antibody heavy chain coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 52, 62, or 83), a T2A peptide cleavage sequence (*e.g.*, the T2A peptide cleavage sequence

of SEQ ID NO: 28), at least a portion of an antibody light chain coding sequence (*e.g.*, the antibody light chain coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 53 or 63), a polyadenylation sequence (*e.g.*, the SV40 polyadenylation sequence of SEQ ID NO: 31), and a 3′ ITR (*e.g.*, the 3′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 18).

**[00190]** In certain embodiments, the foregoing methods employ an rAAV comprising an AAV capsid protein comprising the amino acid sequence of amino acids 138-736 of SEQ ID NO: 13, and an rAAV genome comprising 5′ to 3′ following genetic elements: a 5′ ITR (*e.g.*, the 5′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 14), a transcriptional regulatory element (*e.g.*, a TRE comprising the nucleotide sequence set forth in SEQ ID NO: 27), an intron element (*e.g.*, the intron element comprising the nucleotide sequence set forth in SEQ ID NO: 29), at least a portion of an antibody heavy chain coding sequence (*e.g.*, the antibody heavy chain coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 52, 62, or 83), a T2A peptide cleavage sequence (*e.g.*, the T2A peptide cleavage sequence of SEQ ID NO: 28), at least a portion of an antibody light chain coding sequence (*e.g.*, the antibody light chain coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 53 or 63), a polyadenylation sequence (*e.g.*, the SV40 polyadenylation sequence of SEQ ID NO: 31), and a 3′ ITR (*e.g.*, the 3′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 18).

**[00191]** In certain embodiments, the foregoing methods employ an rAAV comprising an AAV capsid protein comprising the amino acid sequence of SEQ ID NO: 13, and an rAAV genome comprising 5′ to 3′ following genetic elements: a 5′ ITR (*e.g.*, the 5′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 14), a transcriptional regulatory element (*e.g.*, a TRE comprising the nucleotide sequence set forth in SEQ ID NO: 27), an intron element (*e.g.*, the intron element comprising the nucleotide sequence set forth in SEQ ID NO: 29), at least a portion of an antibody heavy chain coding sequence (*e.g.*, the antibody heavy chain coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 52, 62, or 83), a polyadenylation sequence (*e.g.*, the BGH polyadenylation sequence of SEQ ID NO: 33), a transcriptional regulatory element (*e.g.*, a TRE comprising the nucleotide sequence set forth in SEQ ID NO: 67), an intron element (*e.g.*, the intron element comprising the nucleotide sequence set forth in SEQ ID NO: 30 or 61), at least a portion of an antibody light chain coding sequence (*e.g.*, the antibody light chain coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 53 or 63), a polyadenylation sequence (*e.g.*, the SV40 polyadenylation

sequence of SEQ ID NO: 31), and a 3′ ITR (*e.g.*, the 3′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 18).

**[00192]** In certain embodiments, the foregoing methods employ an rAAV comprising an AAV capsid protein comprising the amino acid sequence of SEQ ID NO: 13, and an rAAV genome comprising 5′ to 3′ following genetic elements: a 5′ ITR (*e.g.*, the 5′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 14), a polyadenylation sequence (*e.g.*, the BGH polyadenylation sequence of SEQ ID NO: 33), at least a portion of an antibody heavy chain coding sequence (*e.g.*, the antibody heavy chain coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 52, 62, or 83), an intron element (*e.g.*, the intron element comprising the nucleotide sequence set forth in SEQ ID NO: 29), a transcriptional regulatory element (*e.g.*, a TRE comprising the nucleotide sequence set forth in SEQ ID NO: 27), a stuffer sequence (*e.g.*, a stuffer comprising the nucleotide sequence set forth in SEQ ID NO: 51), a transcriptional regulatory element (*e.g.*, a TRE comprising the nucleotide sequence set forth in SEQ ID NO: 67), an intron element (*e.g.*, the intron element comprising the nucleotide sequence set forth in SEQ ID NO: 30 or 61), at least a portion of an antibody light chain coding sequence (*e.g.*, the antibody light chain coding sequence comprising the nucleotide sequence set forth in SEQ ID NO: 53 or 63), a polyadenylation sequence (*e.g.*, the SV40 polyadenylation sequence of SEQ ID NO: 31), and a 3′ ITR (*e.g.*, the 3′ ITR comprising the nucleotide sequence set forth in SEQ ID NO: 18).

**[00193]** The methods disclosed herein are particularly advantageous in that they are capable of expressing and secreting an antibody into the serum of a subject with high efficiency *in vivo*. In certain embodiments, the serum concentrations of the antibody is at least about 100 μg/mL, at least about 500 μg/mL, at least about 1000 μg/mL, at least about 1500 μg/mL, at least about 2000 μg/mL, at least about 2500 μg/mL, at least about 3000 μg/mL, at least about 3500 μg/mL, at least about 4000 μg/mL, at least about 4500 μg/mL, at least about 5000 μg/mL, at least about 7500 μg/mL, at least about 10000 μg/mL, at least about 15000 μg/mL, at least about 20000 μg/mL, at least about 25000 μg/mL, at least about 30000 μg/mL, at least about 35000 μg/mL, at least about 40000 μg/mL, at least about 45000 μg/mL, at least about 50000 μg/mL, at least about 60000 μg/mL, at least about 70000 μg/mL, at least about 80000 μg/mL, at least about 90000 μg/mL, or at least about 100000 μg/mL. Any methods of determining the expression level or serum concentration of the antibody can be employed including, without limitation, ELISA, Western blotting, immunostaining, and mass spectrometry. In certain embodiments, the serum concentration of the antibody is determined with an anti-human IgG ELISA.

**[00194]** In certain embodiments, transduction of a cell with an AAV composition disclosed herein can be performed as provided herein or by any method of transduction known to one of ordinary skill in the art. In certain embodiments, the cell may be contacted with the AAV at a multiplicity of infection (MOI) of 50,000; 100,000; 150,000; 200,000; 250,000; 300,000; 350,000; 400,000; 450,000; or 500,000, or at any MOI that provides for optimal transduction of the cell.

**[00195]** An AAV composition disclosed herein can be administered to a subject by any appropriate route including, without limitation, intravenous, intraperitoneal, subcutaneous, intramuscular, intranasal, topical or intradermal routes. In certain embodiments, the composition is formulated for administration via intravenous injection or subcutaneous injection.

## IV. AAV Packaging Systems

**[00196]** In another aspect, the instant disclosure provides packaging systems for recombinant preparation of a recombinant adeno-associated virus (rAAV) disclosed herein. Such packaging systems generally comprise: first nucleotide encoding one or more AAV Rep proteins; a second nucleotide encoding a capsid protein of any of the AAVs as disclosed herein; and a third nucleotide sequence comprising any of the rAAV genomes as disclosed herein, wherein the packaging system is operative in a cell for enclosing the rAAV genome in the capsid to form the AAV.

**[00197]** In certain embodiments, the packaging system comprises a first vector comprising the first nucleotide sequence encoding the one or more AAV Rep proteins and the second nucleotide sequence encoding the AAV capsid protein, and a second vector comprising the third nucleotide sequence comprising the rAAV genome. As used in the context of a packaging system as described herein, a "vector" refers to a nucleic acid molecule that is a vehicle for introducing nucleic acids into a cell (*e.g.*, a plasmid, a virus, a cosmid, an artificial chromosome, etc.).

**[00198]** Any AAV Rep protein can be employed in the packaging systems disclosed herein. In certain embodiments of the packaging system, the Rep nucleotide sequence encodes an AAV2 Rep protein. Suitable AAV2 Rep proteins include, without limitation, Rep 78/68 or Rep 68/52. In certain embodiments of the packaging system, the nucleotide sequence encoding the AAV2 Rep protein comprises a nucleotide sequence that encodes a protein having a minimum percent sequence identity to the AAV2 Rep amino acid sequence of SEQ ID NO: 22, wherein the minimum percent sequence identity is at least 70% (*e.g.*, at least 75%, at least

80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%) across the length of the amino acid sequence of the AAV2 Rep protein. In certain embodiments of the packaging system, the AAV2 Rep protein has the amino acid sequence set forth in SEQ ID NO: 22.

**[00199]** In certain embodiments of the packaging system, the packaging system further comprises a fourth nucleotide sequence comprising one or more helper virus genes. In certain embodiments of the packaging system, the packaging system further comprises a third vector, *e.g.*, a helper virus vector, comprising the fourth nucleotide sequence comprising the one or more helper virus genes. The third vector may be an independent third vector, integral with the first vector, or integral with the second vector.

**[00200]** In certain embodiments of the packaging system, the helper virus is selected from the group consisting of adenovirus, herpes virus (including herpes simplex virus (HSV)), poxvirus (such as vaccinia virus), cytomegalovirus (CMV), and baculovirus. In certain embodiments of the packaging system, where the helper virus is adenovirus, the adenovirus genome comprises one or more adenovirus RNA genes selected from the group consisting of El, E2, E4 and VA. In certain embodiments of the packaging system, where the helper virus is HSV, the HSV genome comprises one or more of HSV genes selected from the group consisting of UL5/8/52, ICPO, ICP4, ICP22 and UL30/UL42.

**[00201]** In certain embodiments of the packaging system, the first, second, and/or third vector are contained within one or more plasmids. In certain embodiments, the first vector and the third vector are contained within a first plasmid. In certain embodiments the second vector and the third vector are contained within a second plasmid.

**[00202]** In certain embodiments of the packaging system, the first, second, and/or third vector are contained within one or more recombinant helper viruses. In certain embodiments, the first vector and the third vector are contained within a recombinant helper virus. In certain embodiments, the second vector and the third vector are contained within a recombinant helper virus.

**[00203]** In a further aspect, the disclosure provides a method for recombinant preparation of an AAV as described herein, wherein the method comprises transfecting or transducing a cell with a packaging system as described herein under conditions operative for enclosing the rAAV genome in the capsid to form the rAAV as described herein. Exemplary methods for recombinant preparation of an rAAV include transient transfection (*e.g.*, with one or more transfection plasmids containing a first, and a second, and optionally a third vector as described herein), viral infection (*e.g.* with one or more recombinant helper viruses, such as a

adenovirus, poxvirus (such as vaccinia virus), herpes virus (including HSV, cytomegalovirus, or baculovirus, containing a first, and a second, and optionally a third vector as described herein), and stable producer cell line transfection or infection (*e.g.*, with a stable producer cell, such as a mammalian or insect cell, containing a Rep nucleotide sequence encoding one or more AAV Rep proteins and/or a Cap nucleotide sequence encoding one or more AAV capsid proteins as described herein, and with an rAAV genome as described herein being delivered in the form of a plasmid or a recombinant helper virus).

**[00204]** Accordingly, the instant disclosure provides a packaging system for preparation of a recombinant AAV (rAAV), wherein the packaging system comprises a first nucleotide sequence encoding one or more AAV Rep proteins; a second nucleotide sequence encoding a capsid protein of any one of the AAVs described herein; a third nucleotide sequence comprising an rAAV genome sequence of any one of the AAVs described herein; and optionally a fourth nucleotide sequence comprising one or more helper virus genes.

## V. Examples

**[00205]** The following examples demonstrate the efficient expression of antibodies (e.g., anti-C5 antibodies) in a subject using an rAAV vector as disclosed herein. These examples are offered by way of illustration, and not by way of limitation.

### Example 1: Anti-Complement C5 Antibody rAAV Vectors

**[00206]** This example provides anti-C5 antibody expressing vectors C5Ab01, C5Ab02, C5Ab03, and C5Ab04 for expression of anti-C5 antibodies in a cell (e.g., a human cell or a mouse cell) into which the vector is transduced.

*a) C5Ab01*

**[00207]** Anti-C5 antibody vector C5Ab01, as shown in FIG. 1, comprises, from 5′ to 3′, the following genetic elements: a transcriptional regulatory element comprising an EF1α promoter; a coding sequence encoding a human IgG2 (P1) signal sequence linked to an anti-C5 antibody heavy chain (HC); a nucleic acid sequence encoding a 2A ribosomal skipping peptide; a coding sequence encoding an Igκ (P2) signal sequence linked to an anti-C5 antibody light chain (LC); and an SV40 late polyadenylation sequence (LPA). The nucleic sequences of these elements are set forth in Table 1. This vector is capable of expressing an anti-C5 antibody in a cell (e.g., a human cell or a mouse cell) into which the vector is transduced.

*b) C5Ab02*

**[00208]** Anti-C5 antibody vector C5Ab02, as shown in FIG. 1, comprises, from 5′ to 3′, the following genetic elements: a transcriptional regulatory element comprising the liver-specific LP1 promoter; a coding sequence encoding a human IgG2 (P1) signal sequence linked to an anti-C5 antibody heavy chain (HC); a nucleic acid sequence encoding 2A ribosomal skipping peptide; a coding sequence encoding an Igκ (P2) signal sequence linked to an anti-C5 antibody light chain (LC); and an SV40 late polyadenylation sequence (LPA). The sequences of these elements are set forth in Table 1. This vector is capable of expressing an anti-C5 antibody in a cell (e.g., a human cell or a mouse cell) into which the vector is transduced.

*c) C5Ab03*

**[00209]** Anti-C5 antibody vector C5Ab03, as shown in FIG. 1, comprises, from 5′ to 3′, the following genetic elements: a transcriptional regulatory element comprising the liver-specific LP1 promoter; a coding sequence encoding a human IgG2 (P1) signal sequence linked to an anti-C5 antibody heavy chain (HC); a bovine growth hormone polyadenylation signal (bGHpA); a transcriptional regulatory element comprising the liver-specific DnG promoter; a coding sequence encoding an Igκ (P2) signal sequence linked to an anti-C5 antibody light chain (LC); and an SV40 late polyadenylation sequence (LPA). The sequences of these elements are set forth in Table 1. This vector is capable of expressing an anti-C5 antibody in a cell (e.g., a human cell or a mouse cell) into which the vector is transduced.

*d) C5Ab04*

**[00210]** Anti-C5 antibody vector C5Ab04, as shown in FIG. 1, comprises from 5′ to 3′, the following genetic elements: a bovine growth hormone polyadenylation signal (bGHpA); an anti-C5 antibody heavy chain coding sequence; a coding sequence encoding an anti-C5 antibody heavy chain (HC) linked to a human IgG2 (P1) signal sequence; a transcriptional regulatory element comprising the liver-specific LP1 promoter; a stuffer sequence; a transcriptional regulatory element comprising the liver-specific DnG promoter; a coding sequence encoding an Igκ (P2) signal sequence linked to an anti-C5 antibody light chain (LC); and an SV40 late polyadenylation sequence (LPA). The sequences of these elements are set forth in Table 1. This vector is capable of expressing an anti-C5 antibody in a cell (e.g., a human cell or a mouse cell) into which the vector is transduced.

**Table 1: Genetic elements in anti-C5 antibody expressing vectors C5Ab01, C5Ab02, C5Ab03, and C5Ab04**

| | C5Ab01 | C5Ab02 | C5Ab03 | C5Ab04 |
|---|---|---|---|---|

| **Genetic element (from 5′ to 3′)** | **SEQ ID NO** | | | |
|---|---|---|---|---|
| 5′ ITR element | 14 | 14 | 14 | 14 |
| First Transcriptional regulatory element | 60 | 50 | 50 | 50 |
| Antibody heavy chain signal sequence | 23 | 23 | 23 | 23 |
| Anti-C5 antibody heavy chain coding sequence | 52 | 52 | 52 | 52 |
| First Polyadenylation Sequence | N/A | N/A | 33 | 33 |
| Second Transcriptional regulatory element | N/A | N/A | 43 | 43 |
| Antibody light chain signal sequence | 24 | 24 | 24 | 24 |
| Anti-C5 antibody light chain coding sequence | 53 | 53 | 53 | 53 |
| Second Polyadenylation Sequence | 31 | 31 | 31 | 31 |
| 3′ ITR element | 18 | 18 | 18 | 18 |
| rAAV genome (from promoter to polyA sequence) | 84 | 85 | 86 | 87 |
| rAAV genome (from 5′ ITR to 3′ ITR) | 88 | 89 | 90 | 91 |

**[00211]** The vectors disclosed herein can be packaged in an AAV capsid, including, without limitation, an AAVHSC5, AAVHSC7, AAVHSC8, AAVHSC13, AAVHSC15, or AAVHSC17 capsid.

**Example 2: Expression of Anti-C5 Antibodies in a Mouse Model**

**[00212]** Aberrant or excessive activity of the complement component C5 is associated with several diseases, including paroxysmal nocturnal hemoglobinuria (PNH), neuromyelitis optica spectrum disorder, (NMOSD), and atypical hemolytic uremic syndrome (aHUS). Anti-C5 monoclonal antibodies have been shown to be effective in treating these diseases, but patients often require multiple large doses of the antibody to enjoy the therapeutic benefits. This is, in part, due to the high concentration of C5 protein in the patient's serum. It, therefore, requires high levels anti-C5 antibodies to bind and eliminate enough C5 to produce the required therapeutic effect. These issues may be overcome if a patient is capable of expressing their own anti-C5 antibodies.

**[00213]** To study if sufficiently high levels of anti-C5 antibodies can be expressed in an organism, NOD SCID mice were administered AAV vectors for the expression of said anti-C5 antibodies from the mouse liver. Four separate experiments were performed, testing vectors C5Ab02, C5Ab03, and C5Ab04 (described above), packaged in each of AAVHSC13, AAVHSC15, and AAVHSC17.

**[00214]** Human IgG ELISA Protocol

**[00215]** To evaluate serum human IgG concentration (μg/mL) in the following experiments, the SimpleStep ELISA® kit from Abcam was employed. Briefly, an antibody cocktail was prepared by diluting the capture and detector antibodies in Antibody Diluent CP. To make 3 mL of the antibody cocktail, 300 μL of 10X Capture Antibody and 300 μL 10X Detector Antibody were combined with 2.4 mL Antibody Diluent CP. Standards were subsequently prepared by serial dilution immediately prior to use. Human IgG protein provided in the kit was used for the positive control serial dilution.

**[00216]** To conduct the assay, all reagents were brought to room temperature prior to use. 50 μL of all samples or standards were added to appropriate wells of a microplate. 50 μL of the antibody cocktail was then added to each well. The plate was then sealed and incubated for 40 minutes at room temperature on a plate shaker set to 400 rpm. Each well was then washed with 3 x 350 μL 1X Wash Buffer PT. After the last wash, the plate was inverted and blotted against clean paper towels to remove excess liquid. 100 μL of TMB Development Solution was then added to each well and incubated for about 5 minutes in the dark on a plate shaker set to 400 rpm. Optimal incubation time may vary between 5 and 20 minutes. 100 μL of Stop Solution was added to each well. The plate was shaken on a plate shaker for 1 minute to mix. The optical density (OD) was then read at 450 nm. This was the endpoint reading.

**[00217]** Results

**[00218]** In a first experiment, mice received vector C5Ab04 packaged in the AAVHSC13 or the AAVHSC17 capsid at a dose of 1e13 vgs/kg. Serum samples were taken after 1 week, 3 weeks, 5 weeks, 7 weeks, 9 weeks, 11 weeks, 15 weeks, 19 weeks, and 23 weeks. The serum samples were tested for human IgG concentration (μg/mL) as a readout of anti-C5 antibody levels. Female mice are poor models for AAV-mediated gene transfer, so the data was segregated between male and female mice. As shown in FIG. 2A, mice receiving C5Ab04 packaged in either the AAVHSC13 or the AAVHSC17 capsid demonstrated elevated levels of anti-C5 antibodies over time. FIG. 2B shows the results in FIG. 2A with the Y-axis in a logarithmic scale. In FIGs. 2A-2B, n = 2-3 mice per group.

**[00219]** In a second experiment, mice received vector C5Ab02 packaged in the AAVHSC17 capsid at a dose of 1e13 vgs/kg. Data for male and female mice were segregated and multiple serum samples were taken over a period of 16 weeks. The serum samples were tested for human IgG concentration (μg/mL) as a readout of anti-C5 antibody levels. As shown in FIG. 2C, mice receiving vector C5Ab02 packaged in the AAVHSC17 capsid demonstrated elevated levels of anti-C5 antibodies over time. FIG. 2D shows the results in FIG. 2C with the Y-axis in a logarithmic scale. In FIGs. 2C-2D, n = 3 mice per group.

**[00220]** In a third experiment, mice received vectors C5Ab02, C5Ab03, or C5Ab04, each packaged in the AAVHSC15 or the AAVHSC17 capsid at a dose of 1e13 vgs/kg. Data for male mice is shown and multiple serum samples were taken over a period of 16 weeks. The serum samples were tested for human IgG concentration (μg/mL) as a readout of anti-C5 antibody levels. As shown in FIG. 2E, mice receiving any one of vectors C5Ab02, C5Ab03, or C5Ab04, packaged in either the AAVHSC15 or the AAVHSC17 capsid, demonstrated elevated levels of anti-C5 antibodies over time. FIGs. 2F and 2G (Y-axis in a logarithmic scale) show the results in FIG. 2E presented in line graph format. In FIGs. 2E-2G, n = 3 male mice per group.

**[00221]** In a fourth experiment, mice received vector C5Ab04 packaged in the AAVHSC17 capsid at 5 doses, 5e11 vgs/kg, 5e12 vgs/kg, 1.4e13 vgs/kg, 4.4e13 vgs/kg, and 1.8e14 vgs/kg. Data for male mice is shown and multiple serum samples were taken over a period of 13 weeks. The serum samples were tested for human IgG concentration (μg/mL) as a readout of anti-C5 antibody levels. As shown in the dose response data of FIG. 2H, mice receiving vector C5Ab04 packaged in the AAVHSC17 capsid demonstrated elevated levels of anti-C5 antibodies over time. Moreover, increasing doses of AAVHSC17 lead to corresponding increases in the concentration of anti-C5 antibodies, with doses 1.4e13 vgs/kg, 4.4e13 vgs/kg, and 1.8e14 vgs/kg achieving milligram concentrations of the antibody. FIG. 2I

shows the results in FIG. 2H presented in line graph format with the Y-axis in a logarithmic scale. In FIGs. 2E-2I, n = 3 male mice per group.

**[00222]** The data above was reorganized to compare the efficacy of each vector packaged in each AAVHSC13, AAVHSC15, or AAVHSC17. As shown in FIGs. 3A-3C, C5Ab04 consistently produced higher concentrations of the anti-C5 antibody over time. Moreover, the AAVHSC17 capsid consistently produced higher concentrations of the anti-C5 antibody over time. The combination of C5Ab04 packaged in the AAVHSC17 capsid produced the highest concentrations of the anti-C5 antibody, at approximately 2000 μg/mL, 15 weeks post-delivery to mice.

**[00223]** The data above was also used to predict if the anti-C5 antibody concentrations achieved are consistent with known therapeutic anti-C5 antibodies that are administered directly to patients (*i.e.*, not expressed from a vector in the patient). The pharmacokinetics of commercially available eculizumab and ravulizumab were modeled based on $C_{max}$, $C_{trough}$, and dosing schedule data for the treatment of PNH. The standard deviation ranges were based on the coefficient of variation and number of patients used for these studies (dotted lines in FIGs. 4A and 4B). This modeled data was aligned with data from the above *in vivo* mouse experiments for the AAVHSC13 and AAVHSC17 packaged vectors from the first experiment. The comparison reveals that the therapeutic approach described herein can achieve clinically effective levels of known anti-C5 antibodies, ravulizumab and eculizumab (FIG. 4A). The modeled data was also aligned with data from the above *in vivo* mouse experiments (first experiment: "NOD-SCID, 1E+13 vg/kg"; and fourth experiment: "NOD-SCID 1.8E+14 vg/kg"), as well as data from the HuLiv mouse experiments below (Example 4), for C5Ab04 packaged in the AAVHSC17 capsid (FIG. 4B).

**Example 3: *Ex vivo* Analysis of Anti-C5 Antibodies Expressed in a Mouse Model**

**[00224]** Paroxysmal nocturnal hemoglobinuria (PNH) is characterized by destruction of red blood cells (hemolytic anemia), blood clots (thrombosis), and impaired bone marrow function. To determine if the expressed anti-C5 antibodies are effective at reducing hemolytic anemia, a PNH *ex vivo* hemolysis assay was performed. Human serum, containing human C5, was mixed with serum obtained from the NOD SCID mice treated with AAVHSC17-packaged C5Ab04, and activated sheep red blood cells (RBCs). The % hemolysis was compared against a control anti-C5 biosimilar antibody. As shown in FIG. 5, the serum obtained from the NOD SCID mice treated with either AAVHSC13-packaged C5Ab04 or AAVHSC17-packaged

C5Ab04 was capable of inhibiting hemolysis to a greater extent than the biosimilar control anti-C5 antibody.

**[00225]** A similar *ex vivo* hemolysis assay was performed, comparing the serum obtained from the NOD SCID mice treated with either AAVHSC13-packaged C5Ab04 or AAVHSC17-packaged C5Ab04 at a dose of 1e13 vg/kg with negative control mouse serum (from experiment in FIG. 2A). Data for male and female mice were segregated and multiple serum samples were taken over a period of 9 weeks post-treatment. Human IgG concentration (μg/mL) was measured in serum samples as a readout of anti-C5 antibody levels, and demonstrated elevated levels of anti-C5 antibodies over time in mice treated with C5Ab04 packaged in either the AAVHSC13 or the AAVHSC17 capsid (FIG. 6A). Data presented in FIG. 6A is a subset of data presented in FIGs. 2A and 2B. Serum obtained from the mice treated with C5Ab04 packaged in either the AAVHSC13 or the AAVHSC17 capsid was capable of inhibiting hemolysis (FIG. 6B). FIG. 6C shows the results in FIG. 6B with % hemolysis determined from serum samples obtained out to 19 weeks post-administration, and presented in a line graph. In FIGs. 6A-6C, n = 3 mice per group.

**[00226]** In a second experiment, an *ex vivo* hemolysis assay was performed, comparing the serum obtained from NOD SCID mice treated with AAVHSC17-packaged C5Ab02 at a dose of 1e13 vgs/kg, with negative control mouse serum (as in FIG. 2C). Data for male and female mice were segregated and multiple serum samples were taken over a period of 16 weeks post-treatment. As shown in FIG. 6D, serum obtained from the treated NOD SCID mice were capable of inhibiting hemolysis. In FIG. 6D, n = 3 mice per group.

**[00227]** In a third experiment, an *ex vivo* hemolysis assay was performed, comparing the serum obtained from male NOD SCID mice treated with AAVHSC15 or AAVHSC17-packaged C5Ab02, C5Ab03, or C5Ab04, at a dose of 1e13 vgs/kg, with negative control mouse serum (as in FIG. 2E). Multiple serum samples were taken over a period of 16 weeks post-treatment. As shown in FIG. 6E, serum obtained from the treated NOD SCID mice were capable of inhibiting hemolysis. In FIG. 6E, n = 3 male mice per group.

**[00228]** In a fourth experiment, an *ex vivo* hemolysis assay was performed, comparing the serum obtained from male NOD SCID mice treated with AAVHSC17-packaged C5Ab04 at a dose of 5e11 vgs/kg, 5e12 vgs/kg, 1.4e13 vgs/kg, 4.4e13 vgs/kg, or 1.8e14 vgs/kg, with negative control mouse serum (as in FIG. 2H). Multiple serum samples were taken over a period of 16 weeks post-treatment. As shown in FIG. 6F, serum obtained from the treated NOD SCID mice were capable of inhibiting hemolysis in an rAAV-dose-dependent manner. In FIG. 6F, n = 3 male mice per group.

**[00229]** Hemolysis Assay Protocol

**[00230]** The above referenced hemolysis assay was performed using the following protocol. In a 96-well V-bottom plate, Gelatin Veronal buffer (GVBS, Sigma, Cat#G6514) was mixed with mouse serum in each well (with and without EDTA). 10% Normal Human Serum (NHS, Sigma, Cat#H4522) was then added to all wells. The plate was then incubated at 37° C for 30 minutes. 1 mL of antibody-sensitized sheep erythrocytes (Complement Technology, Inc., Catalog Numbers: B200, B201 and B202) were then added to each well and shaken for about 30 minutes. The plate was then centrifuged at 1000g for 5 minutes. The supernatant was moved to a new plate and read at 540 nm and 615 nm. The 615-nm value was then subtracted from 540 nm value to obtain the final reading. In all reported % hemolysis values, % hemolysis of all samples, including the formulation buffer-only or AAVHSC-treated samples, is reported after normalizing with 100% red blood cell lysis control.

**Example 4: Generation and Characterization of the HuLiv Mouse Model**

**[00231]** To evaluate the functional activity and durability of the therapeutic approach described herein *Fah*$^{-/-}$ *Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ mice on the C57Bl/6 background, commonly referred to as the FRG® Knockout mice, were used as a model for liver humanization. The mice were immunodeficient and lacked the tyrosine catabolic enzyme fumarylacetoacetate hydrolase (Fah). Ablation of mouse hepatocytes was induced by the withdrawal of the protective drug 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC). The mice were then engrafted with human hepatocytes, and a urokinase-expressing adenovirus was administered to enhance repopulation of the human hepatocytes. Engraftment was sustained over the life of the animal with an appropriate regimen of CuRx™ Nitisinone (20-0026) and prophylactic treatment of SMX/TMP antibiotics (20-0037). Resulting HuLiv mouse livers were repopulated with >70% human hepatocytes. These HuLiv mice are described further in Azuma et al. (Nature Biotechnology. 25(8): 903-910 (2007)).

**[00232]** As shown in FIG. 7A and FIG. 7B, the HuLiv mouse produces human C5 at levels comparable to human serum while producing less mouse C5. This HuLiv model allows for the examination of anti-C5 antibody expression by human hepatocytes *in vivo*, and anti-C5 antibody durability in the presence of human C5.

**[00233]** HuLiv mice (n = 2) were administered a single dose of 100 µg of a control anti-C5 biosimilar antibody or administered C5Ab04 packaged in the AAVHSC17 capsid at a dose of 1e13 vgs/kg or 1e14 vgs/kg. Serum antibody concentration was determined at week 1, 3, 5, 7, 9, and 11. As shown in FIG. 8A for weeks 1, 3, and 5, administration of C5Ab04 packaged

in the AAVHSC17 capsid to the HuLiv mice led to substantially higher concentrations of the anti-C5 antibody compared to direct administration of the control anti-C5 antibody. FIG. 8B shows the results in FIG. 8A with serum antibody concentration determined out to week 11 post-administration, and presented in a line graph with the Y-axis in a logarithmic scale. An *ex vivo* hemolysis assay was performed using the same methodology as described above, comparing the serum obtained from the HuLiv mice treated with C5Ab04 packaged in the AAVHSC17 capsid at a dose of 1e13 vgs/kg or 1e14 vgs/kg, with the serum obtained from a HuLiv mouse directly administered a control anti-C5 antibody (biosimilar). Serum samples were taken over a period of 11 weeks post-treatment. As shown in FIG. 8C, serum obtained from the treated HuLiv mice were capable of inhibiting hemolysis. The mice treated with C5Ab04 packaged in AAVHSC17 capsid at a dose of 1e13 vgs/kg or 1e14 vgs/kg showed about 80% protection from hemolysis in the *ex vivo* hemolysis assay. Background residual hemolysis of up to about 20% may be explained by the presence of mouse complement proteins made by a residual population of C57Bl/6 hepatocytes in HuLiv mice. FIG. 8D shows the level of mouse C5 detected via an enzyme-linked immunosorbent assay in serum obtained from the treated HuLiv mice.

**Example 5: Primary Hepatocyte Screening Assay**

**[00234]** In order to rapidly test optimized rAAV vectors, a cell line-based assay was developed to assess antibody production and secretion. Human primary hepatocytes were selected as the closest match to the *in vivo* experiments. Plateable human hepatocytes (Cat. # HUCPG) and plateable C57BL/6 mouse hepatocytes (Cat. # MBCP01) from Lonza were used.

**[00235]** Approximately 500,000 human hepatocytes or 250,000 mouse hepatocytes were plated, followed by incubation with approximately 300,000 MOI of AAVHSC15 or AAVHSC17 packaged with C5Ab02, C5Ab03, or C5Ab04. Culture media was then collected on day 7 after viral addition and analyzed by western blot and human IgG ELISA. As shown in FIG. 9A and FIG. 9B, abundant levels of the anti-C5 antibodies were detected.

* * *

**[00236]** The invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

**[00237]** All references (e.g., publications or patents or patent applications) cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual reference (e.g., publication or patent or patent application) was specifically and individually indicated to be incorporated by reference in its entirety for all purposes. Other embodiments are within the following claims.

**We claim:**

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

(a) a first expression cassette comprising, from 5′ to 3′,

a first liver-specific transcriptional regulatory element,

a first coding sequence encoding a first polypeptide comprising an antibody heavy chain operably linked to a first signal sequence, and

a first polyadenylation sequence; and

(b) a second expression cassette comprising, from 5′ to 3′,

a second liver-specific transcriptional regulatory element,

a second coding sequence encoding a second polypeptide comprising an antibody light chain operably linked to a second signal sequence, and

a second polyadenylation sequence,

wherein expression of the first and second coding sequences produces an antibody comprising the antibody heavy chain and the antibody light chain.

2. The rAAV genome of claim 1, wherein the first and/or second transcriptional regulatory element comprise a promoter element selected from the group consisting of human albumin promoter, a human transthyretin (TTR) promoter, a human thyroxine binding globulin (TBG) promoter, a human ApoH promoter, a human SERPINA1 (hAAT) promoter, and a hepatic specific regulatory module thereof, such as a human ApoE/C-I hepatic control region (HCR) 1 or 2.

3. The rAAV genome of claim 1 or 2, wherein the first and/or second transcriptional regulatory element comprise a promoter element comprising a nucleic acid sequence at least 90% identical to a sequence selected from the group consisting of SEQ ID NO: 25, 27, 66, 68, 69, 116, and 117.

4. The rAAV genome of any one of claims 1-3, wherein the transcriptional regulatory element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 27.

5. The rAAV genome of any one of claims 1-4, wherein the transcriptional regulatory element comprises the nucleotide sequence set forth in SEQ ID NO: 27.

6. The rAAV genome of any one of claims 1-5, wherein the nucleotide sequence of the transcriptional regulatory element consists of the nucleotide sequence set forth in SEQ ID NO: 27.

7. The rAAV genome of any one of claims 1-3, wherein the transcriptional regulatory element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 67.

8. The rAAV genome of any one of claims 1-4, wherein the transcriptional regulatory element comprises the nucleotide sequence set forth in SEQ ID NO: 67.

9. The rAAV genome of any one of claims 1-5, wherein the nucleotide sequence of the transcriptional regulatory element consists of the nucleotide sequence set forth in SEQ ID NO: 67.

10. The rAAV genome of any one of claims 1-9, wherein the first and/or second expression cassette further comprises an intron element positioned 5′ to the first and/or second coding sequence and 3′ to the transcriptional regulatory element.

11. The rAAV genome of claim 10, wherein the intron element is an exogenous intron element, optionally wherein the exogenous intron element is an SV40 intron element or a minute virus of mouse (MVM) intron element.

12. The rAAV genome of claim 11, wherein the SV40 intron element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 29.

13. The rAAV genome of claim 11 or 12, wherein the SV40 intron element comprises the nucleotide sequence set forth in SEQ ID NO: 29.

14. The rAAV genome of any one of claims 11-13, wherein the nucleotide sequence of the SV40 intron element consists of the nucleotide sequence set forth in SEQ ID NO: 29.

15. The rAAV genome of any one of claims 11-14, wherein the MVM intron element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 30.

16. The rAAV genome of any one of claims 11-14, wherein the MVM intron element comprises the nucleotide sequence set forth in SEQ ID NO: 30.

17. The rAAV genome of any one of claims 11-16, wherein the nucleotide sequence of the MVM intron element consists of the nucleotide sequence set forth in SEQ ID NO: 30.

18. The rAAV genome of any one of claims 1-17, wherein the first and second transcriptional regulatory element are identical.

19. The rAAV genome of any one of claims 1-18, wherein the first transcriptional regulatory element comprises an HCR 1 element, a hAAT promoter, and an SV40 intron element, and the second transcriptional regulatory element comprises a SERPINA1 hepatic specific regulatory module, a TTR promoter, and an MVM intron element.

20. The rAAV genome of any one of claims 1-19, wherein the first transcriptional regulatory element comprises the nucleic acid sequence of SEQ ID NO: 50 and the second transcriptional regulatory element comprises the nucleic acid sequence of SEQ ID NO: 43.

21. The rAAV genome of any one of claims 1-20, wherein the first and/or second expression cassette further comprises a polyadenylation sequence 3′ to the first and/or second coding sequence.

22. The rAAV genome of claim 21, wherein the polyadenylation sequence is an exogenous polyadenylation sequence, optionally wherein the exogenous polyadenylation sequence is an SV40 polyadenylation sequence, or a bovine growth hormone (BGH) polyadenylation sequence.

23. The rAAV genome of claim 22, wherein the SV40 polyadenylation sequence comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 31.

24. The rAAV genome of claim 22 or 23, wherein the SV40 polyadenylation sequence comprises the nucleotide sequence set forth in SEQ ID NO: 31.

25. The rAAV genome of any one of claims 22-24, wherein the nucleotide sequence of the SV40 polyadenylation sequence consists of the nucleotide sequence set forth in SEQ ID NO: 31.

26. The rAAV genome of any one of claims 22-25, wherein the BGH polyadenylation sequence comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 33.

27. The rAAV genome of any one of claims 22-26, wherein the BGH polyadenylation sequence comprises the nucleotide sequence set forth in SEQ ID NO: 33.

28. The rAAV genome of any one of claims 22-27, wherein the nucleotide sequence of the BGH polyadenylation sequence consists of the nucleotide sequence set forth in SEQ ID NO: 33.

29. The rAAV genome of any one of claims 21-28, wherein the first and second expression cassette comprise identical polyadenylation sequences.

30. The rAAV genome of any one of claims 21-29, wherein the first expression cassette comprises the SV40 polyadenylation sequence.

31. The rAAV genome of any one of claims 21-30, wherein the second expression cassette comprises the BGH polyadenylation sequence.

32. The rAAV genome of any one of claims 21-31, wherein the first polyadenylation sequence comprises the nucleic acid sequence of SEQ ID NO: 31 and the second polyadenylation sequence comprises the nucleic acid sequence of SEQ ID NO: 33.

33. The rAAV genome of any one of claims 1-32, wherein the first and second expression cassettes are in the same orientation in the rAAV genome.

34. The rAAV genome of any one of claims 1-32, wherein the first and second expression cassettes are in opposite orientations in the rAAV genome.

35. The rAAV genome of any of the preceding claims, wherein the first and second expression cassettes are in opposite orientations, with the first and second polyadenylation sequences distally positioned in the rAAV genome.

36. The rAAV genome of claim 35, wherein the rAAV genome further comprises a stuffer sequence interposed between the first and second transcriptional regulatory elements.

37. The rAAV genome of claim 36, wherein the stuffer sequence comprises a beta globin polyadenylation sequence.

38. The rAAV genome of claim 37, wherein the beta globin polyadenylation sequence comprises the nucleic acid sequence of SEQ ID NO: 51.

39. The rAAV genome of claim 35, wherein the rAAV genome comprises from 5′ to 3′:

(a) the first polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 33;
(b) the first coding sequence;
(c) the first liver-specific transcriptional regulatory element comprising the nucleic acid sequence of SEQ ID NO: 27;
(d) a stuffer sequence comprising the nucleic acid sequence of SEQ ID NO: 51;
(e) the second liver-specific transcriptional regulatory element comprising the nucleic acid sequence of SEQ ID NO: 67;
(f) the second coding sequence; and
(g) the second transcriptional polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 31.

40. The rAAV genome of claim 35, wherein the rAAV genome comprises from 5′ to 3′: the reverse complement of the first expression cassette; a stuffer sequence; and the second expression cassette.

41. The rAAV genome of claim 40, wherein:

(a) the first expression cassette comprises, from 5′ to 3′:

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 27,

the first coding sequence,

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 33;

(b) the stuffer sequence comprising a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and

(c) the second expression cassette comprising, from 5′ to 3′,

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 67,

the second coding sequence,

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 31.

42. The rAAV genome of claim 40, wherein:

(a) the first expression cassette comprises, from 5′ to 3′:

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 67,

the first coding sequence,

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 31;

(b) the stuffer sequence comprising a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and

(c) the second expression cassette comprising, from 5′ to 3′,

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 27,

the second coding sequence,

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 33.

43 The rAAV genome of claim 40, wherein:

(a) the first expression cassette comprises, from 5′ to 3′:

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 25,

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 26,

the first coding sequence,

the first polyadenylation sequence;

(b) the stuffer sequence comprising a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and

(c) the second expression cassette comprising, from 5′ to 3′,

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 119,

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 45,

the second coding sequence,

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 31.

44. The rAAV genome of claim 40, wherein:

(a) the first expression cassette comprises, from 5′ to 3′:

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 119,

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 45,

the first coding sequence,

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 31;

(b) the stuffer sequence comprising a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and

(c) the second expression cassette comprising, from 5′ to 3′,

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 25,

a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 26,

the second coding sequence,

the first polyadenylation sequence.

45. The rAAV genome of claim 40, wherein:

(a) the first expression cassette comprises, from 5′ to 3′:

the nucleotide sequence set forth in SEQ ID NO: 27,

the first coding sequence,

the nucleotide sequence set forth in SEQ ID NO: 33;

(b) the stuffer sequence comprising the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and

(c) the second expression cassette comprising, from 5′ to 3′,

the nucleotide sequence set forth in SEQ ID NO: 67,

the second coding sequence,

the nucleotide sequence set forth in SEQ ID NO: 31.

46. The rAAV genome of claim 40, wherein:

(a) the first expression cassette comprises, from 5′ to 3′:

the nucleotide sequence set forth in SEQ ID NO: 67,

the first coding sequence,

the nucleotide sequence set forth in SEQ ID NO: 31;

(b) the stuffer sequence comprising the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and

(c) the second expression cassette comprising, from 5′ to 3′,

the nucleotide sequence set forth in SEQ ID NO: 27,

the second coding sequence,

the nucleotide sequence set forth in SEQ ID NO: 33.

47. The rAAV genome of claim 40, wherein:

(a) the first expression cassette comprises, from 5′ to 3′:

the nucleotide sequence set forth in SEQ ID NO: 25,

the nucleotide sequence set forth in SEQ ID NO: 26,

the first coding sequence,

the first polyadenylation sequence;

(b) the stuffer sequence comprising the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and

(c) the second expression cassette comprising, from 5′ to 3′,

the nucleotide sequence set forth in SEQ ID NO: 119,

the nucleotide sequence set forth in SEQ ID NO: 45,

the second coding sequence,

the nucleotide sequence set forth in SEQ ID NO: 31.

48. The rAAV genome of claim 40, wherein:

(a) the first expression cassette comprises, from 5′ to 3′:

the nucleotide sequence set forth in SEQ ID NO: 119,

the nucleotide sequence set forth in SEQ ID NO: 45,

the first coding sequence,

the nucleotide sequence set forth in SEQ ID NO: 31;

(b) the stuffer sequence comprising the nucleotide sequence set forth in SEQ ID NO: 51 or the reverse complement thereof; and

(c) the second expression cassette comprising, from 5′ to 3′,

the nucleotide sequence set forth in SEQ ID NO: 25,

the nucleotide sequence set forth in SEQ ID NO: 26,

the second coding sequence,

the first polyadenylation sequence.

49. An rAAV genome comprising a bicistronic expression cassette comprising, from 5′ to 3′:

(a) a liver-specific transcriptional regulatory element; a first coding sequence encoding a first polypeptide comprising an antibody heavy chain operably linked to a first signal sequence; a ribosomal skipping sequence encoding a ribosomal skipping peptide; a second coding sequence encoding a second polypeptide comprising an antibody light chain operably linked to a second signal sequence; and a polyadenylation sequence; or

(b) a liver-specific transcriptional regulatory element; a second coding sequence encoding a second polypeptide comprising an antibody light chain operably linked to a second signal sequence; a ribosomal skipping sequence encoding a ribosomal skipping peptide; a first coding sequence encoding a first polypeptide comprising an antibody heavy chain operably linked to a first signal sequence; and a polyadenylation sequence,

wherein expression of the bicistronic expression cassette produces an antibody comprising the antibody heavy chain and the antibody light chain.

50. The rAAV genome of claim 49, wherein transcriptional regulatory element comprises a promoter element selected from the group consisting of human albumin promoter, a human transthyretin (TTR) promoter, the human thyroxine binding globulin (TBG) promoter, a human ApoH promoter, a human SERPINA1 (hAAT) promoter, and a hepatic specific regulatory module thereof, such as a human ApoE/C-I hepatic control region (HCR) 1 or 2.

51. The rAAV genome of claim 49 or 50, wherein the transcriptional regulatory element comprises a promoter element comprising a nucleic acid sequence at least 90% identical to a sequence selected from the group consisting of SEQ ID NO: 25, 27, 66, 68, 69, 116, and 117.

52. The rAAV genome of any one of claims 49-51, wherein the transcriptional regulatory element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 27.

53. The rAAV genome of any one of claims 49-52, wherein the transcriptional regulatory element comprises the nucleotide sequence set forth in SEQ ID NO: 27.

54. The rAAV genome of any one of claims 49-53, wherein the nucleotide sequence of the transcriptional regulatory element consists of the nucleotide sequence set forth in SEQ ID NO: 27.

55. The rAAV genome of claim 49 or 50, wherein the transcriptional regulatory element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 67.

56. The rAAV genome of claim 55, wherein the transcriptional regulatory element comprises the nucleotide sequence set forth in SEQ ID NO: 67.

57. The rAAV genome of claim 55, wherein the nucleotide sequence of the transcriptional regulatory element consists of the nucleotide sequence set forth in SEQ ID NO: 67.

58. The rAAV genome of any one of claims 49-57, wherein the bicistronic expression cassette further comprises an intron element positioned 5′ to the first and/or second coding sequence and 3′ to the transcriptional regulatory element.

59. The rAAV genome of claim 58, wherein the intron element is an exogenous intron element, optionally wherein the exogenous intron element is an SV40 intron element or a minute virus of mouse (MVM) intron element.

60. The rAAV genome of claim 59, wherein the SV40 intron element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 29.

61. The rAAV genome of claim 59 or 60, wherein the SV40 intron element comprises the nucleotide sequence set forth in SEQ ID NO: 29.

62. The rAAV genome of any one of claims 59-61, wherein the nucleotide sequence of the SV40 intron element consists of the nucleotide sequence set forth in SEQ ID NO: 29.

63. The rAAV genome of claim 59, wherein the MVM intron element comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 30.

64. The rAAV genome of claim 59, wherein the MVM intron element comprises the nucleotide sequence set forth in SEQ ID NO: 30.

65. The rAAV genome of claim 59, wherein the nucleotide sequence of the MVM intron element consists of the nucleotide sequence set forth in SEQ ID NO: 30.

66. The rAAV genome of any one of claims 49-65, wherein the transcriptional regulatory element comprises:

(a) an HCR 1 element, a hAAT promoter, and an SV40 intron element; or

(b) a SERPINA1 hepatic specific regulatory module, a TTR promoter, and an MVM intron element.

67. The rAAV genome of any one of claims 49-66, wherein the transcriptional regulatory element comprises the nucleic acid sequence of SEQ ID NO: 50, or the nucleic acid sequence of SEQ ID NO: 43.

68. The rAAV genome of any one of claims 49-67, wherein the polyadenylation sequence is an exogenous polyadenylation sequence, optionally wherein the exogenous polyadenylation sequence is an SV40 polyadenylation sequence, or a bovine growth hormone (BGH) polyadenylation sequence.

69. The rAAV genome of claim 68, wherein the SV40 polyadenylation sequence comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 31.

70. The rAAV genome of claim 68 or 69, wherein the SV40 polyadenylation sequence comprises the nucleotide sequence set forth in SEQ ID NO: 31.

71. The rAAV genome of any one of claims 68-70, wherein the nucleotide sequence of the SV40 polyadenylation sequence consists of the nucleotide sequence set forth in SEQ ID NO: 31.

72. The rAAV genome of any one of claims 68-71, wherein the BGH polyadenylation sequence comprises a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO: 33.

73. The rAAV genome of any one of claims 68-72, wherein the BGH polyadenylation sequence comprises the nucleotide sequence set forth in SEQ ID NO: 33.

74. The rAAV genome of any one of claims 68-73, wherein the nucleotide sequence of the BGH polyadenylation sequence consists of the nucleotide sequence set forth in SEQ ID NO: 33.

75. The rAAV genome of any one of claims 1-74, wherein the first and/or second signal sequence is a naturally occurring signal sequence.

76. The rAAV genome of any one of claims 1-74, wherein the first and/or second signal sequence is an antibody signal sequence, optionally a human IgG2 or IgK signal sequence.

77. The rAAV genome of any one of claims 1-74, wherein the first and/or second signal sequence is a non-naturally occurring signal sequence.

78. The rAAV genome of any one of claims 1-74, wherein the first and/or second signal sequence comprises the amino acid sequence of SEQ ID NO: 80.

79. The rAAV genome of any one of claims 1-74, wherein the first and/or second signal sequence comprises the amino acid sequence of SEQ ID NO: 81.

80. The rAAV genome of any one of claims 1-79, wherein the first signal sequence comprises the amino acid sequence of SEQ ID NO: 80, and the second signal sequence comprises the amino acid sequence of SEQ ID NO: 81.

81. The rAAV genome of any one of claims 1-80, wherein the first and/or second coding sequence comprises any one of the nucleic acid sequences set forth in SEQ ID NOs: 23, 96, 102, or 108.

82. The rAAV genome of any one of claims 1-80, wherein the first and/or second coding sequence comprises any one of the nucleic acid sequences set forth in SEQ ID NOs: 24, 99, 105, 111, or 130.

83. The rAAV genome of any one of claims 1-82, wherein the first coding sequence comprises any one of the nucleic acid sequences set forth in SEQ ID NOs: 23, 96, 102, or 108 and the second coding sequence comprises any one of the nucleic acid sequences set forth in SEQ ID NOs: 24, 99, 105, 111, or 130.

84. The rAAV genome of any one of claims 1-83, wherein the antibody specifically binds to complement C5.

85. The rAAV genome of any one of claims 1-84, wherein the antibody heavy chain comprises the amino acid sequence of SEQ ID NO: 64.

86. The rAAV genome of any one of claims 1-84, wherein the antibody heavy chain comprises the amino acid sequence of SEQ ID NO: 82.

87. The rAAV genome of any one of claims 1-84, wherein the antibody light chain comprises the amino acid sequence of SEQ ID NO: 77.

88. The rAAV genome of any one of claims 1-84, wherein the first and/or second coding sequence has been optimized for expression in human cells.

89. The rAAV genome of any one of claims 1-84, wherein the first coding sequence comprises any one of the nucleic acid sequences set forth in SEQ ID NO: 52, 113, 114, or 115.

90. The rAAV genome of any one of claims 1-84, wherein the first coding sequence comprises any one of the nucleic acid sequences set forth in SEQ ID NOs: 83, 94, 95, 101, or 107.

91. The rAAV genome of any one of claims 1-84, wherein the second coding sequence comprises any one of the nucleic acid sequences set forth in SEQ ID NOs: 53, 98, 104, 110, or 131.

92. The rAAV genome of any one of claims 1-91, wherein the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 53.

93. The rAAV genome of any one of claims 1-91, wherein the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 63.

94. The rAAV genome of any one of claims 1-91, wherein the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 98.

95. The rAAV genome of any one of claims 1-91, wherein the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 99.

96. The rAAV genome of any one of claims 1-91, wherein the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 100.

97. The rAAV genome of any one of claims 1-91, wherein the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 104.

98. The rAAV genome of any one of claims 1-91, wherein the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 105.

99. The rAAV genome of any one of claims 1-91, wherein the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 106.

100. The rAAV genome of any one of claims 1-91, wherein the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 110.

101. The rAAV genome of any one of claims 1-91, wherein the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95,

96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 111.

102. The rAAV genome of any one of claims 1-91, wherein the first coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 52, 62, 83, 94, 95, 96, 97, 101, 102, 103, 107, 108, 109, 113, 114, and 115, and the second coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 112.

103. The rAAV genome of any one of claims 1-102, wherein the rAAV genome is a single stranded rAAV genome.

104. The rAAV genome of any one of claims 1-103, wherein the rAAV genome is a self-complementary rAAV genome.

105. The rAAV genome of any one of claims 1-104, wherein the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 84.

106. The rAAV genome of any one of claims 1-104, wherein the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 85.

107. The rAAV genome of any one of claims 1-104, wherein the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 86.

108. The rAAV genome of any one of claims 1-104, wherein the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 87.

109. The rAAV genome of any one of claims 1-108, wherein the rAAV genome further comprises a 5′ inverted terminal repeat (5′ ITR) nucleotide sequence 5′ to the first polyadenylation sequence, and a 3′ inverted terminal repeat (3′ ITR) nucleotide sequence 3′ to the second polyadenylation sequence.

110. The rAAV genome of claim 109, wherein the 5′ ITR nucleotide sequence is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence set forth in SEQ ID NO: 14, and/or the 3′ ITR nucleotide

sequence is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence set forth in SEQ ID NO: 18.

111. The rAAV genome of any one of claims 1-110, wherein the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 88.

112. The rAAV genome of any one of claims 1-110, wherein the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 89.

113. The rAAV genome of any one of claims 1-110, wherein the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 90.

114. The rAAV genome of any one of claims 1-110, wherein the rAAV genome comprises the nucleic acid sequence of SEQ ID NO: 91.

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

(a) an AAV capsid comprising an AAV capsid protein; and

(b) an rAAV genome of any one of the preceding claims.

116. The rAAV of claim 115, wherein the capsid protein is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, and AAV9.

117. The rAAV of claim 115 or 116, wherein the AAV capsid protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of amino acids 203-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

118. The rAAV of any one of claims 115-117, wherein: the amino acid in the capsid protein corresponding to amino acid 206 of SEQ ID NO: 16 is C; the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H; the amino acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q; the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A; the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N; the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S; the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I; the amino acid in the capsid protein

corresponding to amino acid 505 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 590 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G or Y; the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M; the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K; the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C; or, the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G.

119. The rAAV of claim 118, wherein:

(a) the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G, and the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G;

(b) the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H, the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M;

(c) the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R;

(d) the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A, and the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; or

(e) the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C.

120. The rAAV of claim 118, wherein the capsid protein comprises the amino acid sequence of amino acids 203-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

121. The rAAV of any one of claims 115-120, wherein the AAV capsid protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of amino acids 138-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

122. The rAAV of claim 121, wherein: the amino acid in the capsid protein corresponding to amino acid 151 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 160 of SEQ ID NO: 16 is D; the amino acid in the capsid protein corresponding to amino acid 206 of SEQ ID NO: 16 is C; the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H; the amino acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q; the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A; the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N; the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S; the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I; the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 590 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G or Y; the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M; the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K; the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C; or, the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G.

123. The rAAV of claim 122, wherein:

(a) the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G, and the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G;

(b) the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H, the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M;

(c) the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R;
(d) the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A, and the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; or
(e) the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C.

124. The rAAV of claim 122, wherein the capsid protein comprises the amino acid sequence of amino acids 138-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 15, 16, or 17.

125. The rAAV of any one of claims 115-124, wherein the AAV capsid protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of amino acids 1-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

126. The rAAV of claim 125, wherein: the amino acid in the capsid protein corresponding to amino acid 2 of SEQ ID NO: 16 is T; the amino acid in the capsid protein corresponding to amino acid 65 of SEQ ID NO: 16 is I; the amino acid in the capsid protein corresponding to amino acid 68 of SEQ ID NO: 16 is V; the amino acid in the capsid protein corresponding to amino acid 77 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 119 of SEQ ID NO: 16 is L; the amino acid in the capsid protein corresponding to amino acid 151 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 160 of SEQ ID NO: 16 is D; the amino acid in the capsid protein corresponding to amino acid 206 of SEQ ID NO: 16 is C; the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H; the amino acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q; the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A; the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N; the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S; the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I; the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to

amino acid 590 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G or Y; the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M; the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K; the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C; or, the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G.

127. The rAAV of claim 126, wherein:

(a) the amino acid in the capsid protein corresponding to amino acid 2 of SEQ ID NO: 16 is T, and the amino acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q;

(b) the amino acid in the capsid protein corresponding to amino acid 65 of SEQ ID NO: 16 is I, and the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is Y;

(c) the amino acid in the capsid protein corresponding to amino acid 77 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K;

(d) the amino acid in the capsid protein corresponding to amino acid 119 of SEQ ID NO: 16 is L, and the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S;

(e) the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G, and the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G;

(f) the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H, the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M;

(g) the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R;

(h) the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A, and the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; or

(i) the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C.

128. The rAAV of claim 127, wherein the capsid protein comprises the amino acid sequence of amino acids 1-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

129. A polynucleotide comprising the nucleic acid sequence set forth in SEQ ID NOs: 85-93.

130. A pharmaceutical composition comprising an rAAV of any one of claims 115-128, or the polynucleotide of claim 129.

131. A packaging system for preparation of an rAAV, wherein the packaging system comprises:

(a) a first nucleotide sequence encoding one or more AAV Rep proteins;

(b) a second nucleotide sequence encoding a capsid protein of the rAAV of any one of claims 115-128; and

(c) a third nucleotide sequence comprising an rAAV genome sequence of the rAAV of any one of claims 1-114.

132. The packaging system of claim 131, wherein the packaging system comprises a first vector comprising the first nucleotide sequence and the second nucleotide sequence, and a second vector comprising the third nucleotide sequence.

133. The packaging system of claim 131 or 132, further comprising a fourth nucleotide sequence comprising one or more helper virus genes.

134. The packaging system of claim 133, wherein the fourth nucleotide sequence is comprised within a third vector.

135. The packaging system of claim 133 or 134, wherein the fourth nucleotide sequence comprises one or more genes from a virus selected from the group consisting of adenovirus, herpes virus, vaccinia virus, and cytomegalovirus (CMV).

136. The packaging system of any one of claims 131-135, wherein the first vector, second vector, and/or the third vector is a plasmid.

137. A method for recombinant preparation of an rAAV, the method comprising introducing the packaging system of any one of claims 131-135 into a cell under conditions whereby the rAAV is produced.

138. The rAAV of any one of claims 115-138, the pharmaceutical composition of claim 130, or the polynucleotide of claim 129, for use as a medicament.

139. The rAAV of any one of claims 115-138, the pharmaceutical composition of claim 130, or the polynucleotide of claim 129, for use in the treatment of complement C5-associated disease.

140. The rAAV of any one of claims 115-138, the pharmaceutical composition of claim 130, or the polynucleotide of claim 129, for use in a method of treating a subject having a complement C5-associated disease, the method comprising administering to the subject an effective amount of the rAAV, the pharmaceutical composition, or the polynucleotide.

141. A method of producing an antibody in a subject, the method comprising administering to the subject the pharmaceutical composition of claim 130.

142. The method of claim 141, wherein the pharmaceutical composition is administered intravenously.

143. A method of treating a complement C5-associated disease in a subject in need thereof, the method comprising administering to the subject an effective amount of the rAAV of any one of claims 115-128, the pharmaceutical composition of claim 130, or the polynucleotide of claim 129.

144. The method of claim 143, wherein the complement C5-associated disease is selected from the group consisting of geographic atrophy (GA), Guillain-Barré syndrome, myasthenia gravis, systemic lupus erythematous (SLE) nephritis, proliferative nephritis, asthma, rheumatoid arthritis, sepsis, paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), and age-related macular degeneration (AMD).

145. The method of claim 143 or 144, wherein the rAAV, the pharmaceutical composition, or the polynucleotide is administered intravenously.

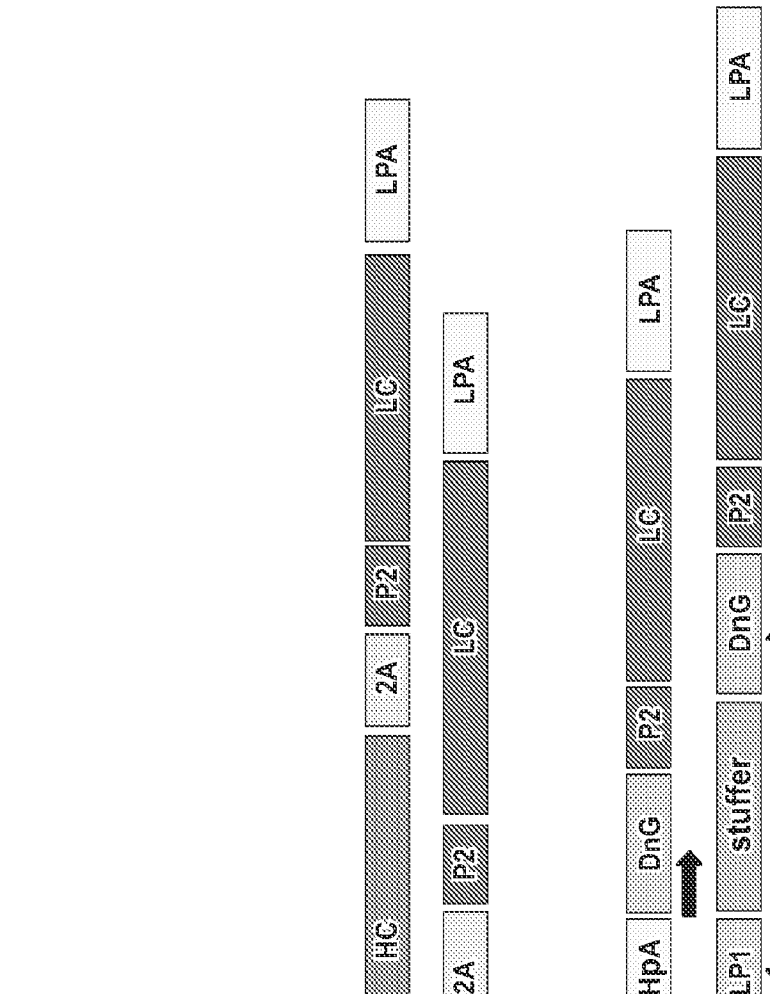
Single Promoter:
C5Ab01
4642bp
EF1α
P1
HC
2A
P2
LC
LPA
C5Ab02
3375bp
LP1
P1
HC
2A
P2
LC
LPA
Dual Liver Specific Promoters:
C5Ab03
3863bp
LP1
P1
HC
bGHpA
DnG
P2
LC
LPA
C5Ab04
4483bp
bGHpA
HC
P1
LP1
stuffer
DnG
P2
LC
LPA

FIG. 1

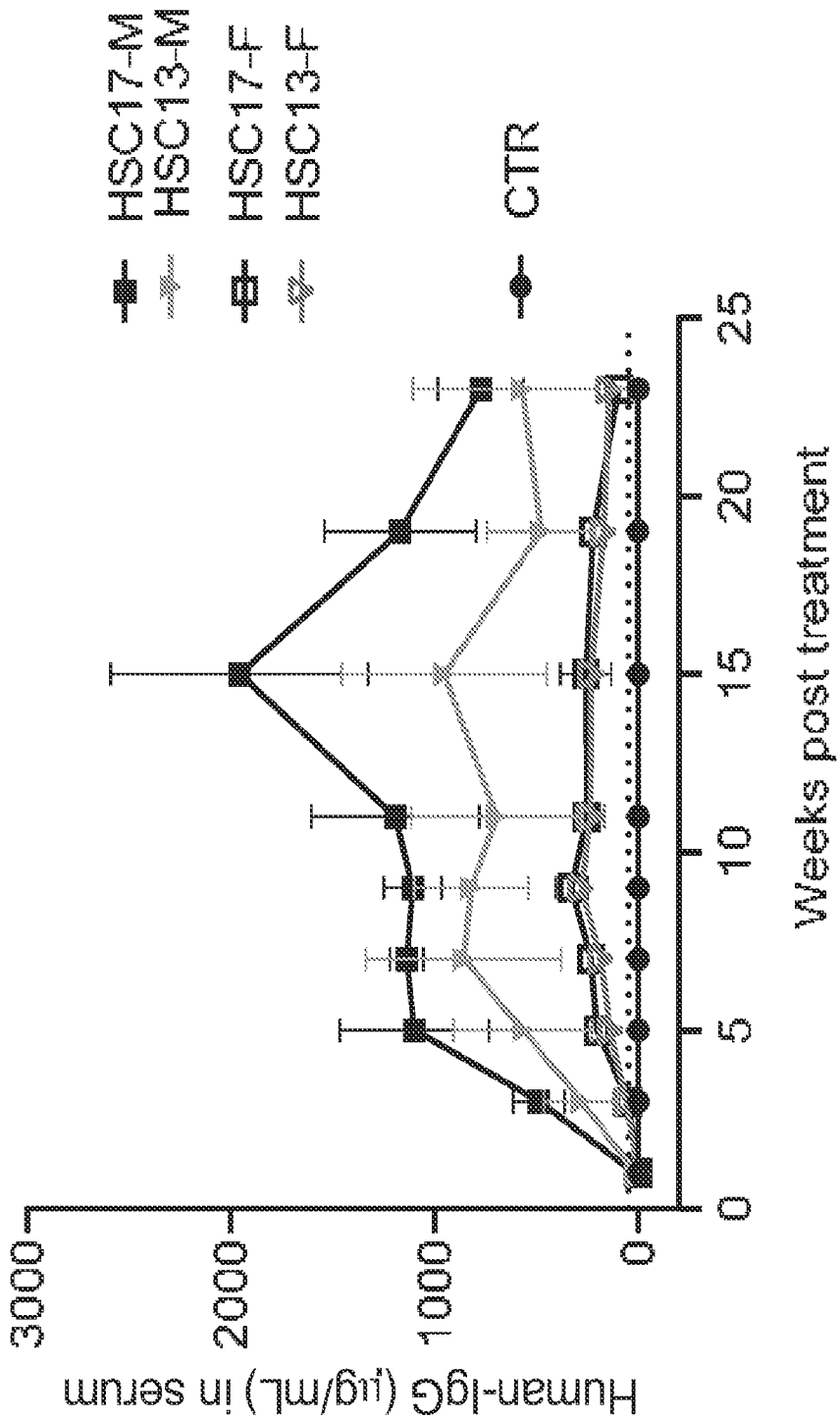
HSC17-M
HSC13-M
HSC17-F
HSC13-F
CTR
Human-IgG (µg/mL) in serum
3000
2000
1000
0
0
5
10
15
20
25
Weeks post treatment

FIG. 2A

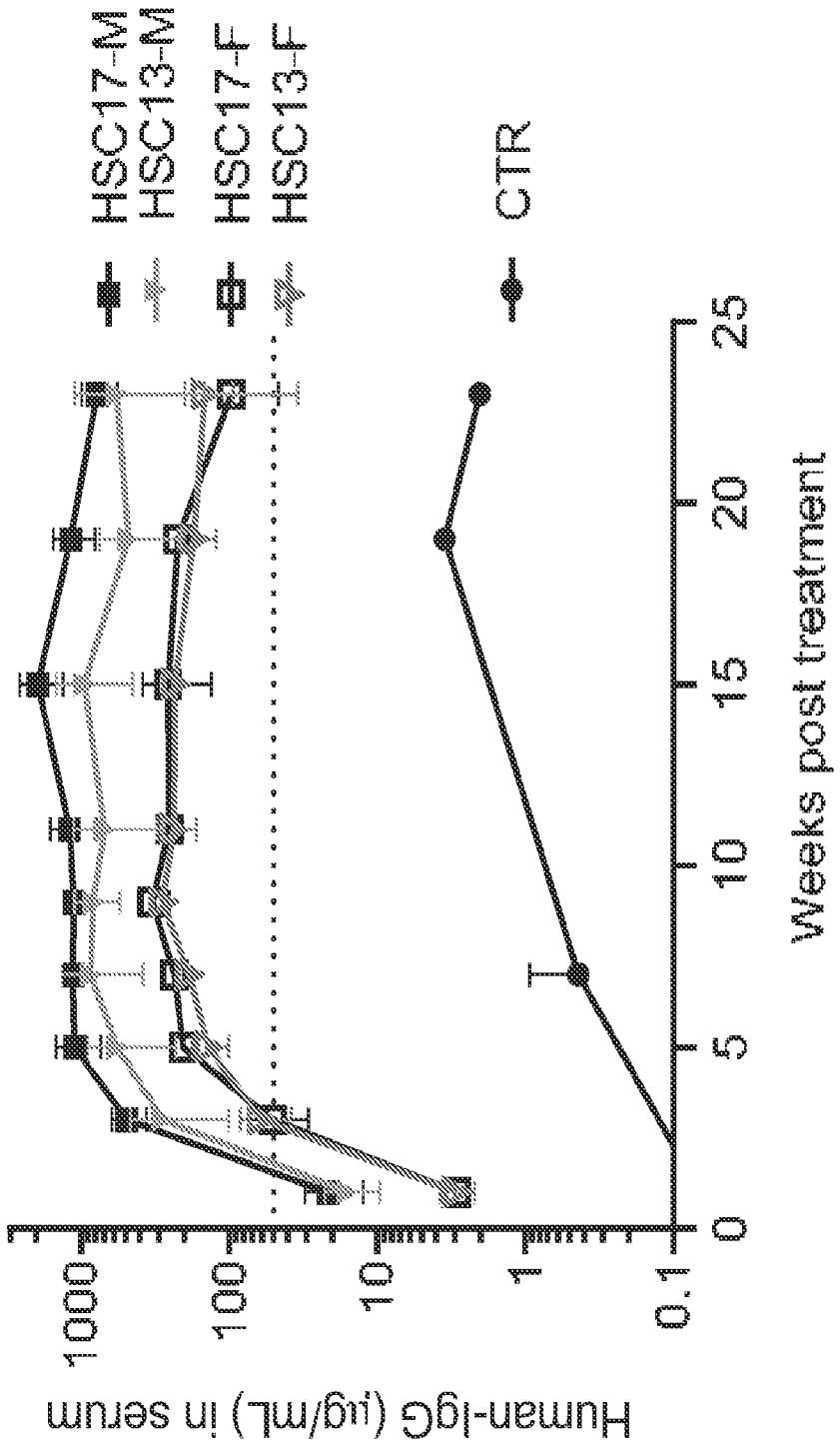
HSC17-M
HSC13-M
HSC17-F
HSC13-F
CTR
Human-IgG (μg/mL) in serum
1000
100
10
1
0.1
0
5
10
15
20
25
Weeks post treatment

FIG. 2B

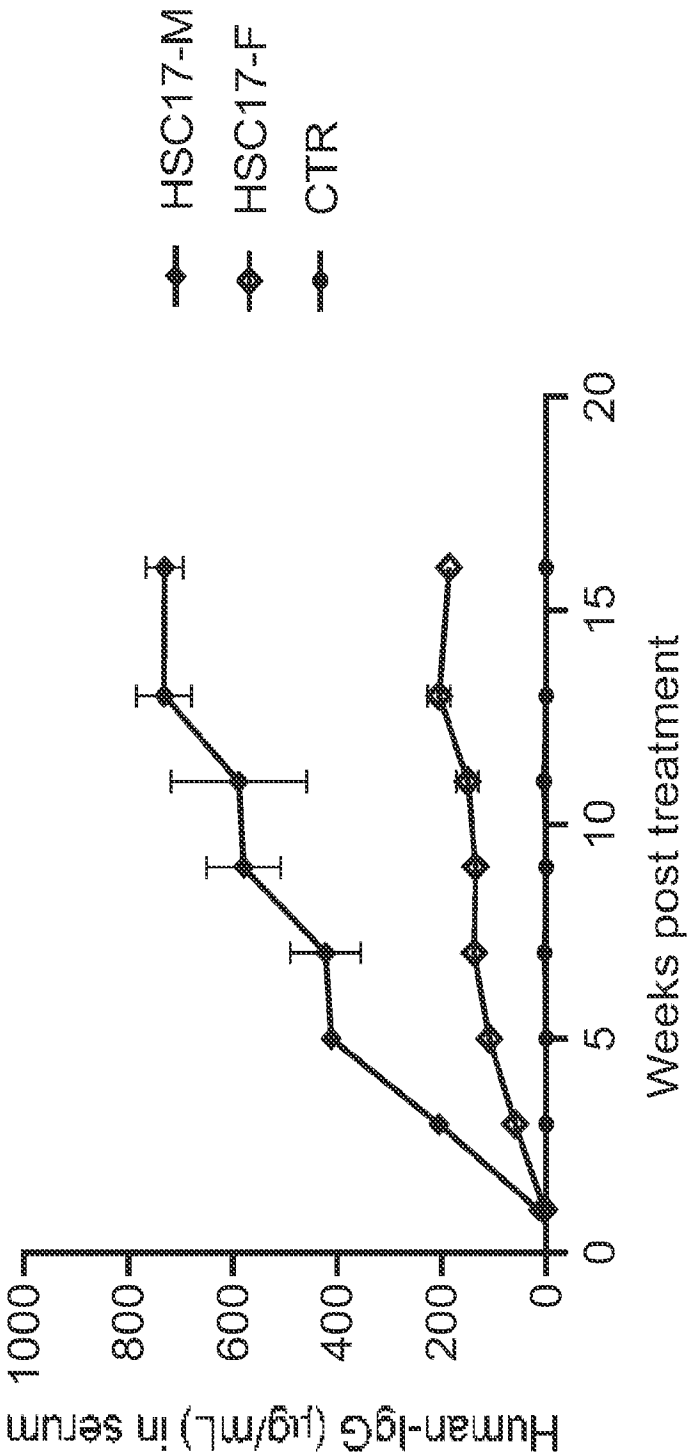
HSC17-M
HSC17-F
CTR
Human-IgG (µg/mL) in serum
1000
800
600
400
200
0
0
5
10
15
20
Weeks post treatment

FIG. 2C

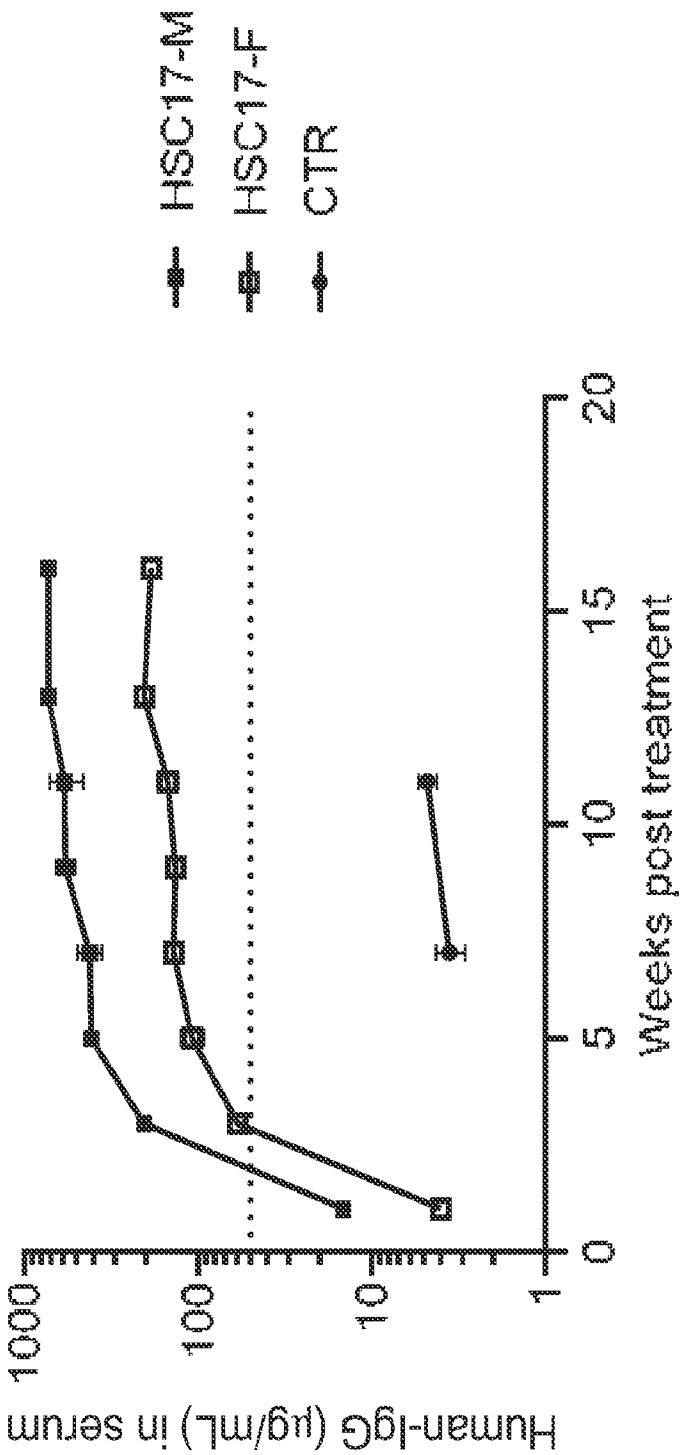
HSC17-M
HSC17-F
CTR
Human-IgG (µg/mL) in serum
1000
100
10
1
0
5
10
15
20
Weeks post treatment

FIG. 2D

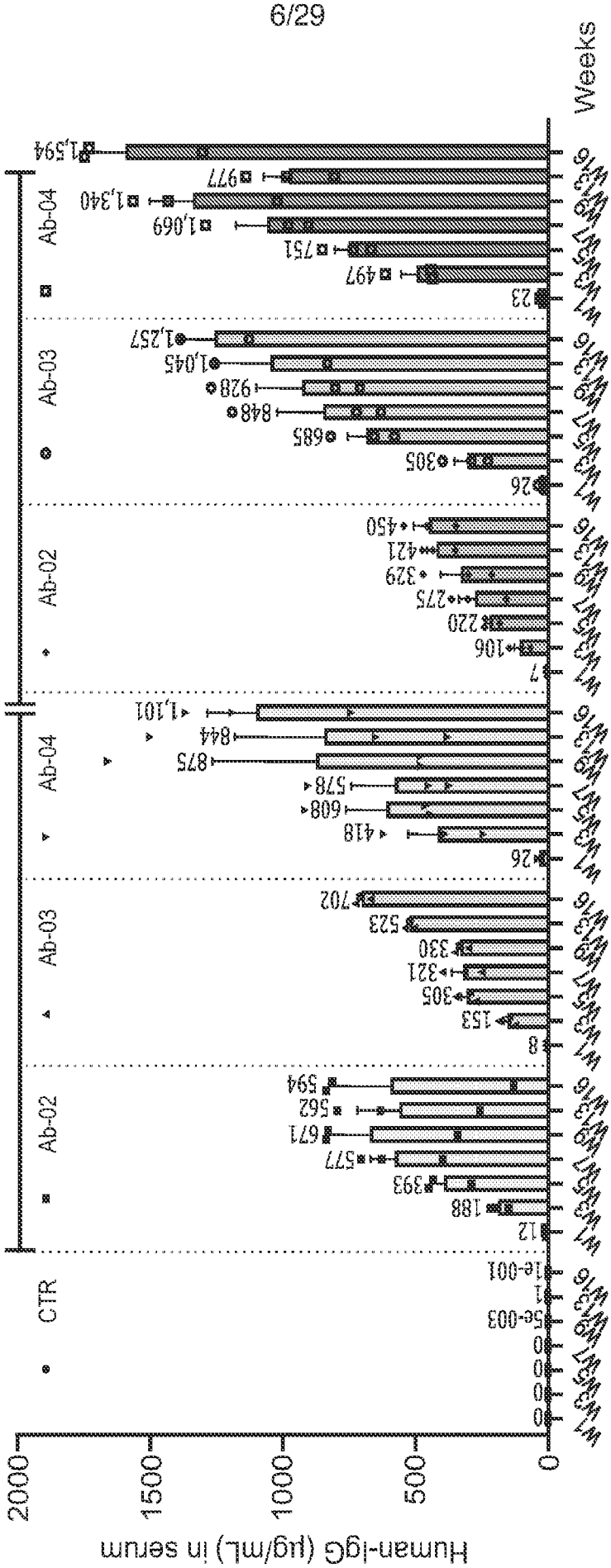
Human-IgG (µg/mL) in serum
2000
1500
1000
500
0
Weeks
AAVHSC15
AAVHSC17
CTR
Ab-02
Ab-03
Ab-04
Ab-02
Ab-03
Ab-04
1e-001
5e-003
0
0
0
0
12
188
393
577
671
562
594
8
153
305
321
330
523
702
26
418
608
578
875
844
1,101
7
106
220
275
329
421
450
26
305
685
848
928
1,045
1,257
23
497
751
1,069
1,340
977
1,594
W1
W3
W5
W7
W9
W13
W16

FIG. 2E

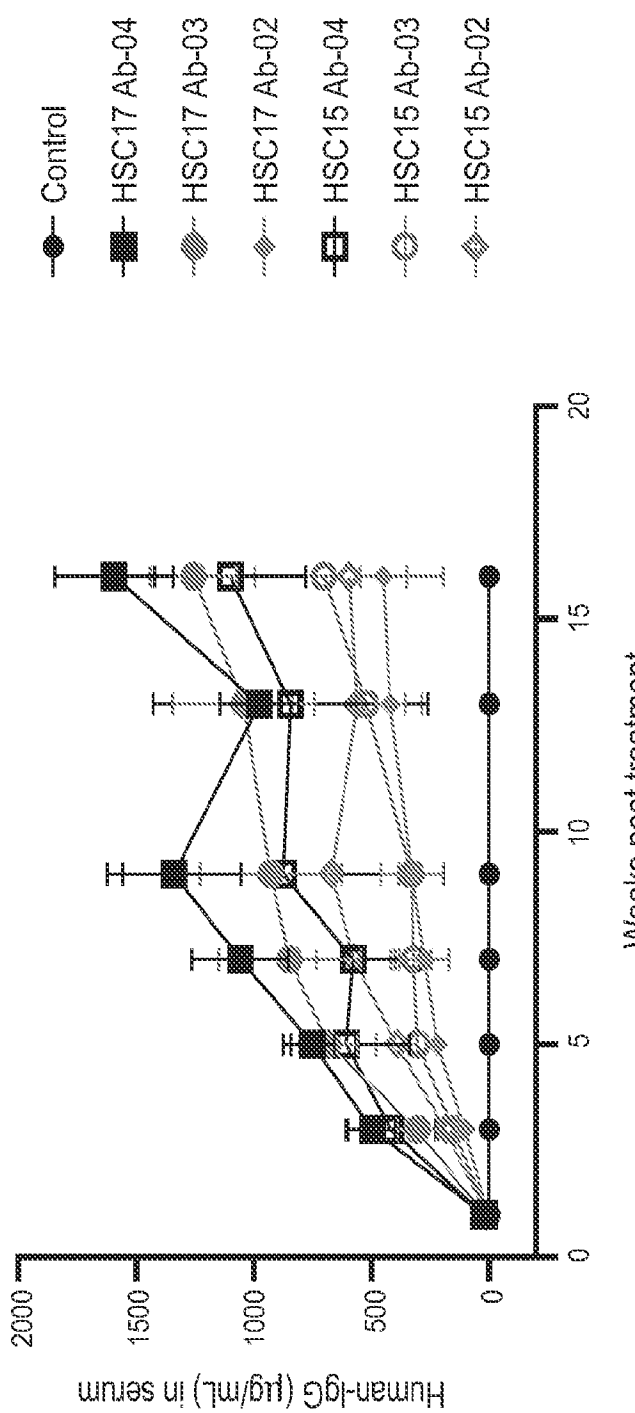
Control
HSC17 Ab-04
HSC17 Ab-03
HSC17 Ab-02
HSC15 Ab-04
HSC15 Ab-03
HSC15 Ab-02
Human-IgG (µg/mL) in serum
2000
1500
1000
500
0
0
5
10
15
20
Weeks post treatment

FIG. 2F

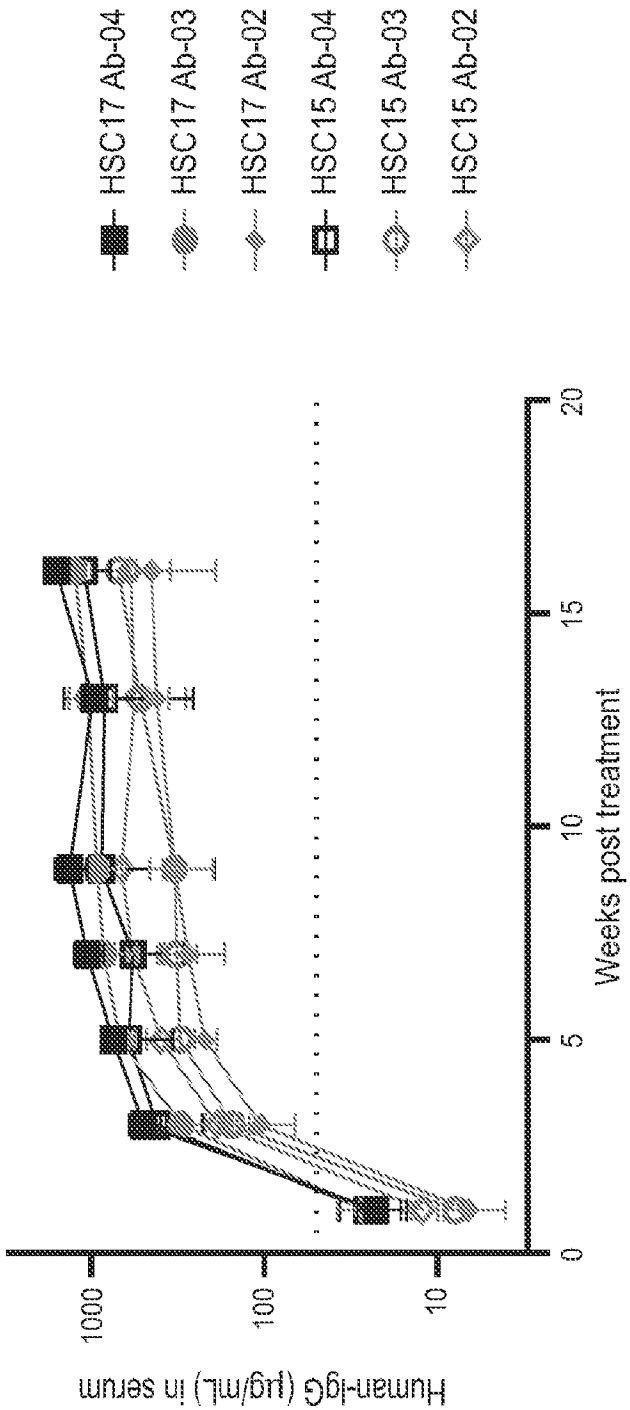
HSC17 Ab-04
HSC17 Ab-03
HSC17 Ab-02
HSC15 Ab-04
HSC15 Ab-03
HSC15 Ab-02
Human-IgG (µg/mL) in serum
1000
100
10
0
5
10
15
20
Weeks post treatment

FIG. 2G

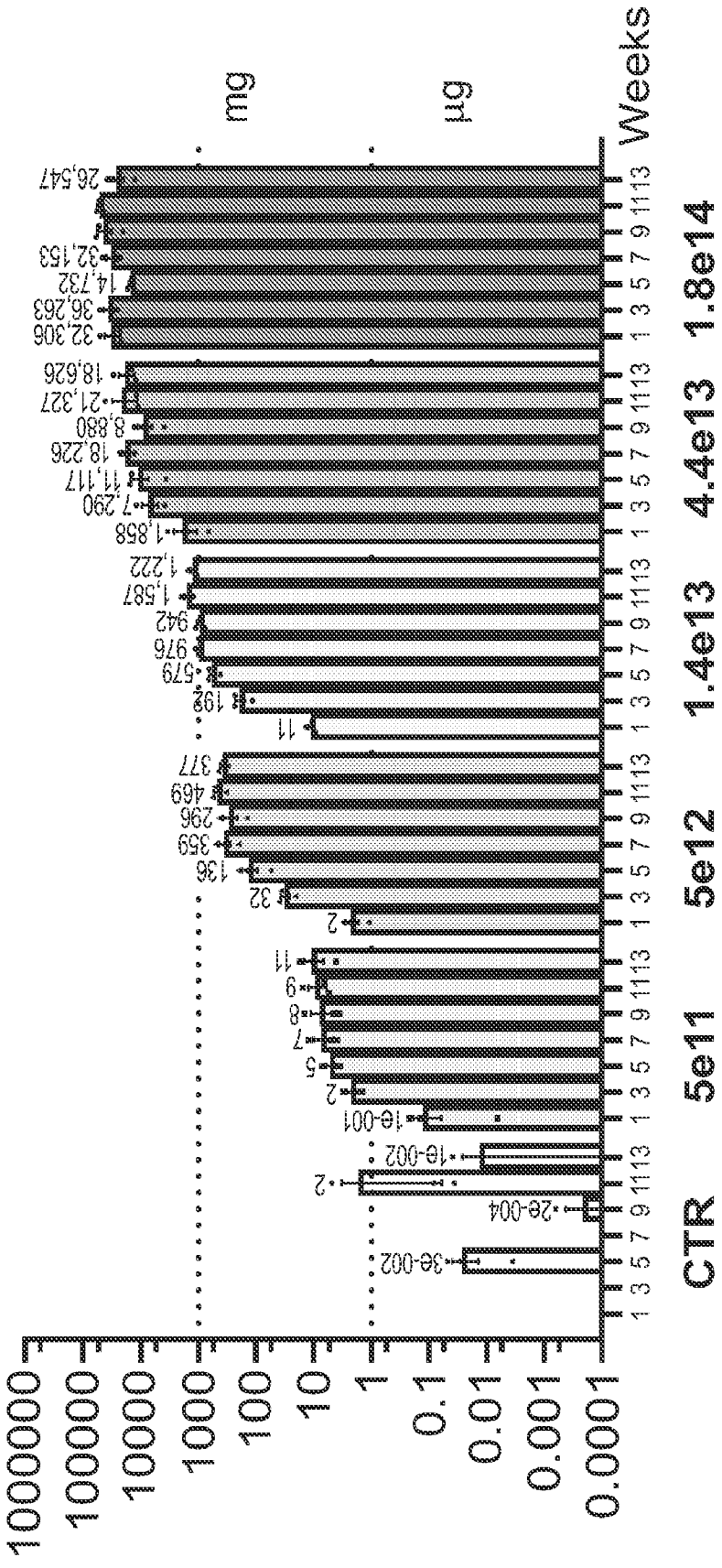
Ab.Liv.01.20
Human-IgG (µg/mL) in serum
1000000
100000
10000
1000
100
10
1
0.1
0.01
0.001
0.0001
mg
µg
Weeks
1 3 5 7 9 11 13
CTR
5e11
5e12
1.4e13
4.4e13
1.8e14
3e-002
2e-004
2
1e-002
1e-001
2
5
7
8
9
11
2
32
136
359
296
469
377
11
192
579
976
942
1,587
1,222
1,858
7,290
11,117
18,226
8,880
21,327
18,626
32,306
36,263
14,732
32,153
26,547

FIG. 2H

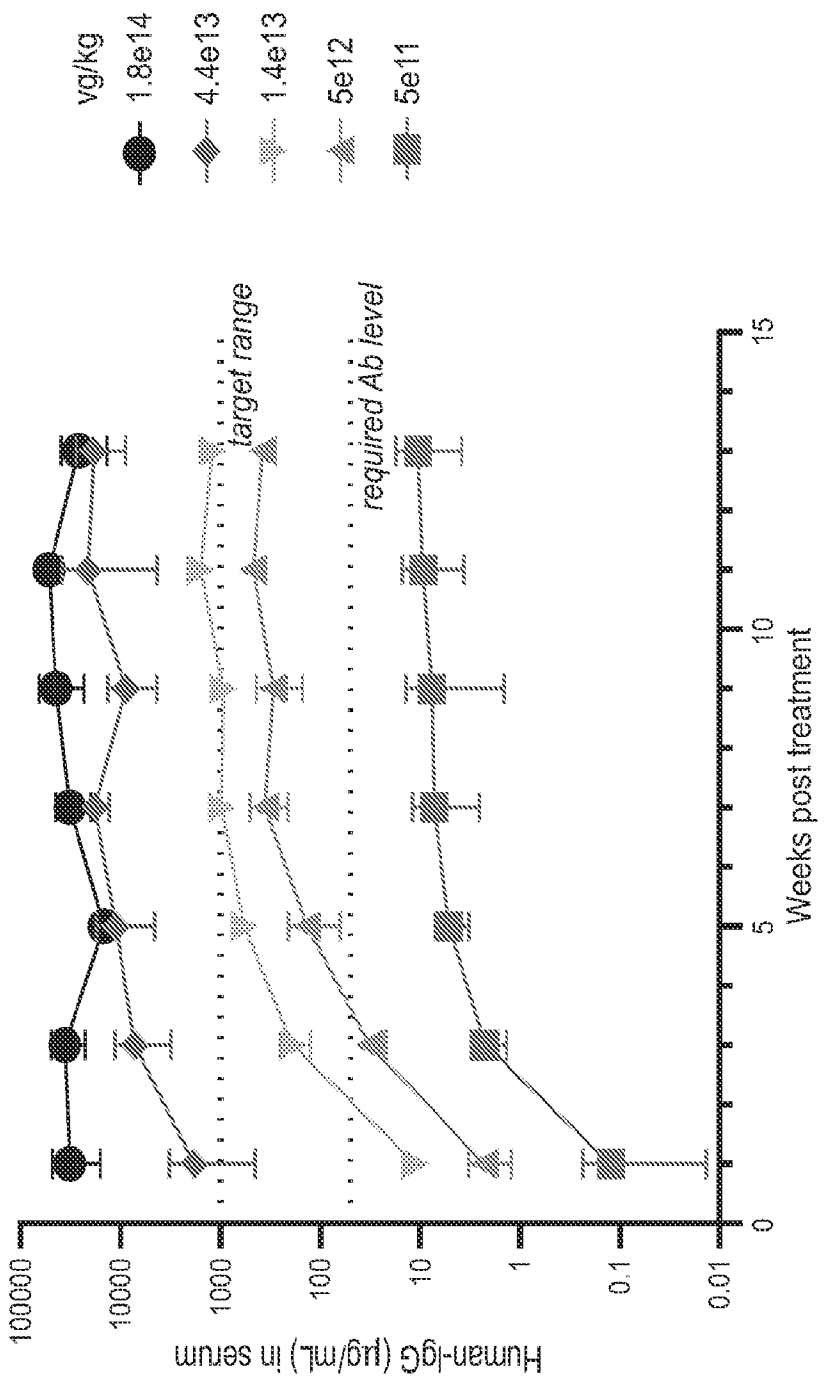
vg/kg
1.8e14
4.4e13
1.4e13
5e12
5e11
target range
required Ab level
Human-IgG (µg/mL) in serum
100000
10000
1000
100
10
1
0.1
0.01
0
5
10
15
Weeks post treatment

FIG. 2I

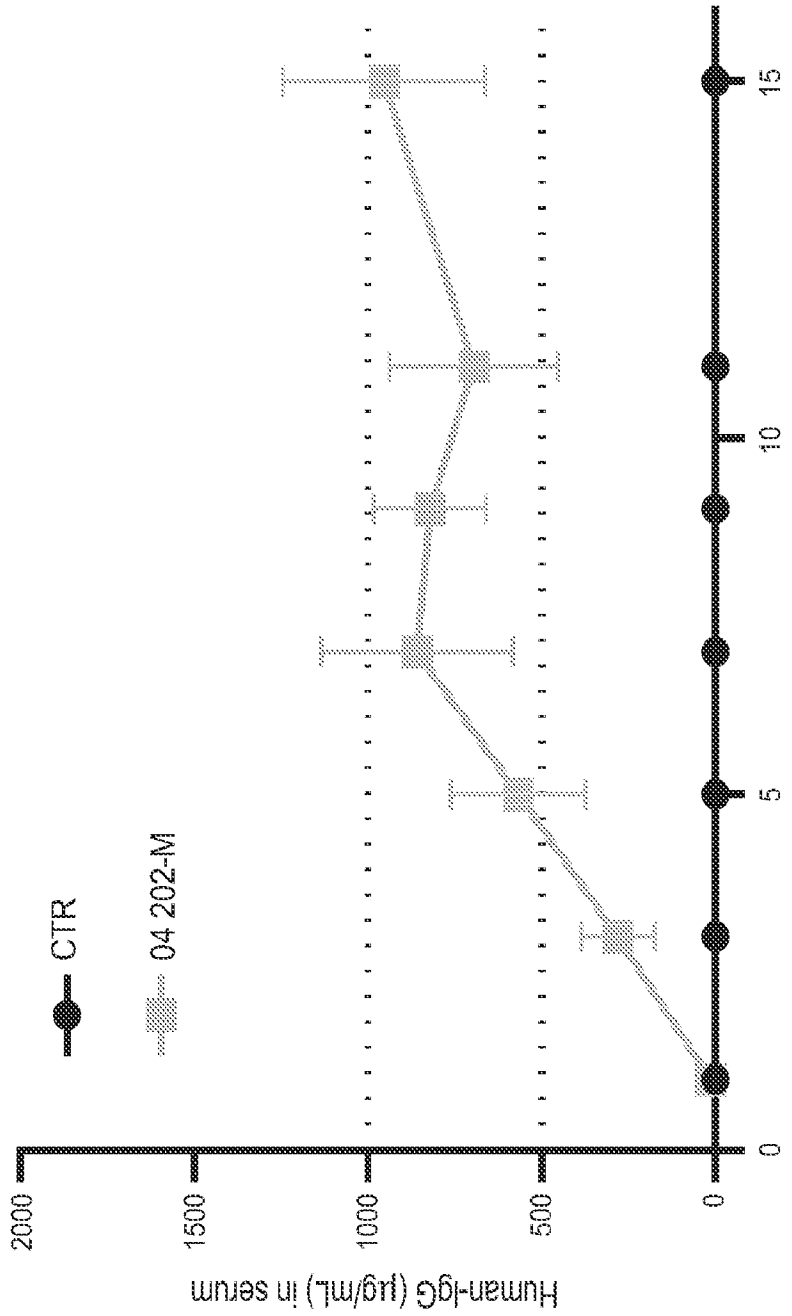
AAVHSC13
CTR
04 202-M
Human-IgG (µg/mL) in serum
2000
1500
1000
500
0
0
5
10
15
Weeks post treatment

FIG. 3A

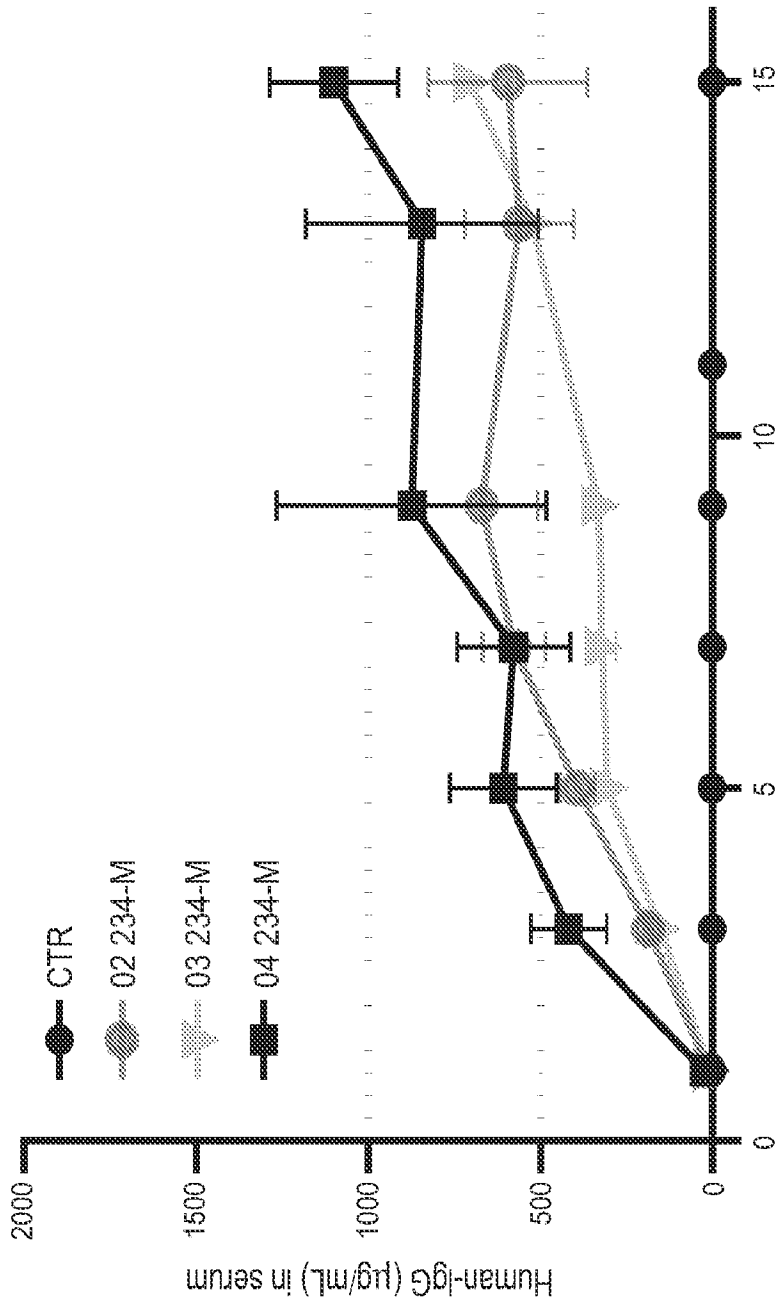
AAVHSC15
CTR
02 234-M
03 234-M
04 234-M
Human-IgG (µg/mL) in serum
2000
1500
1000
500
0
0
5
10
15
Weeks post treatment

FIG. 3B

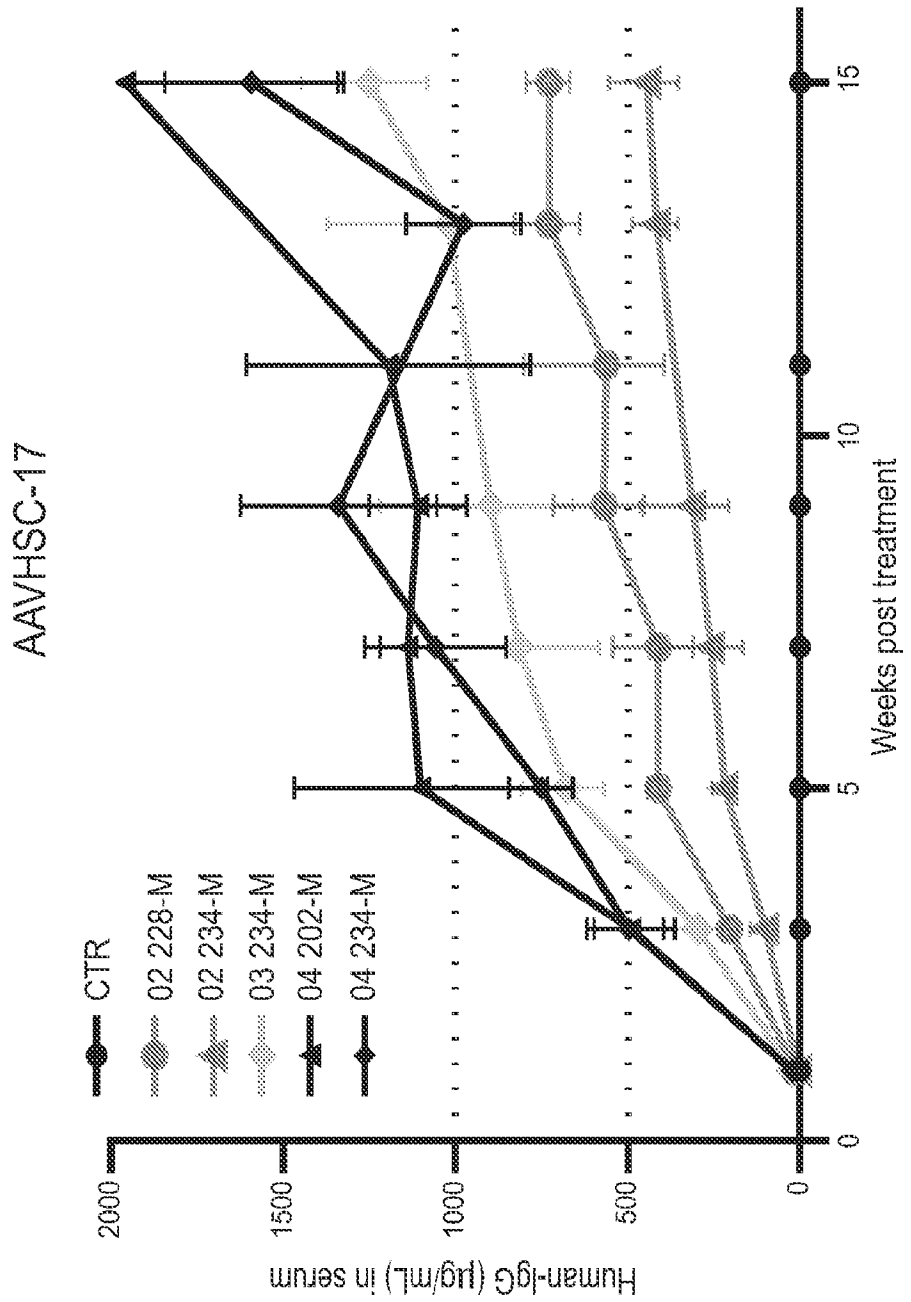
AAVHSC-17
CTR
02 228-M
02 234-M
03 234-M
04 202-M
04 234-M
Human-IgG (µg/mL) in serum
2000
1500
1000
500
0
0
5
10
15
Weeks post treatment

FIG. 3C

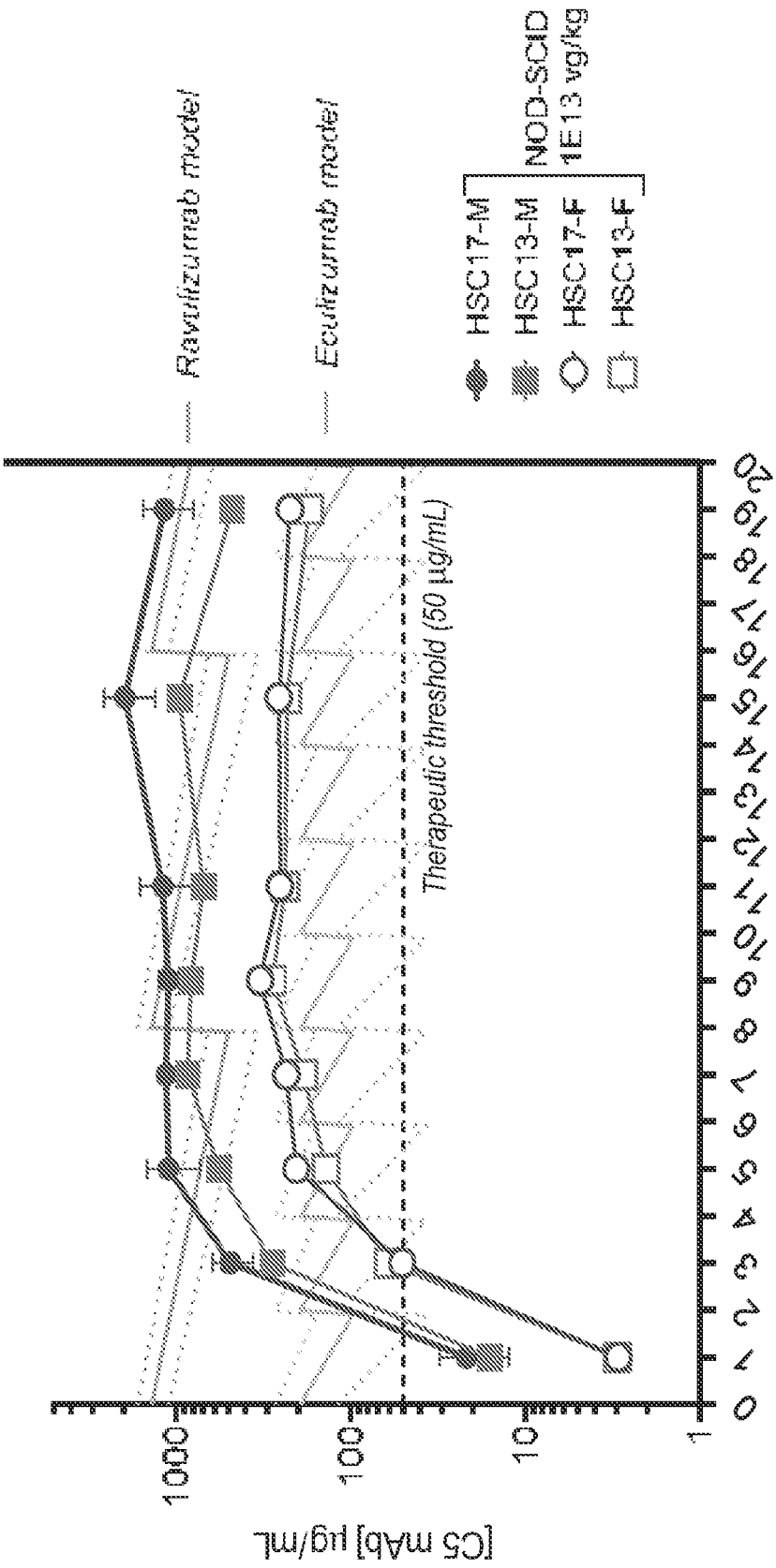
Rituximab model
Ecuclizumab model
HSC17-M
HSC13-M
HSC17-F
HSC13-F
NOD-SCID
1E13 vg/kg
Therapeutic threshold (50 µg/mL)
[C5 mAb] µg/mL
1000
100
10
1
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Weeks Post Treatment

FIG. 4A

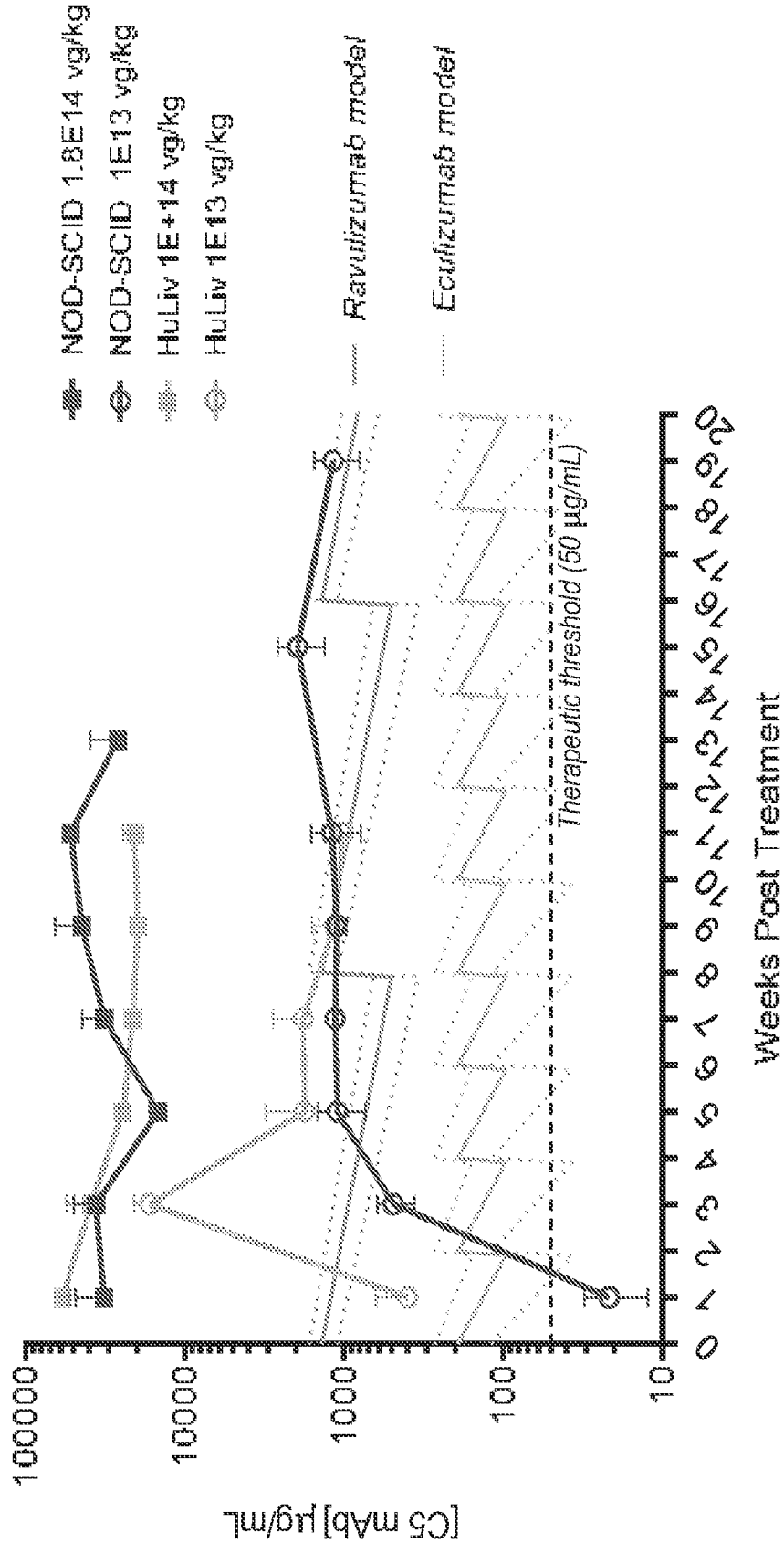
NOD-SCID 1.8E14 vg/kg
NOD-SCID 1E13 vg/kg
HuLiv 1E+14 vg/kg
HuLiv 1E13 vg/kg
Ravulizumab model
Eculizumab model
Therapeutic threshold (50 µg/mL)
[C5 mAb] µg/mL
100000
10000
1000
100
10
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Weeks Post Treatment

FIG. 4B

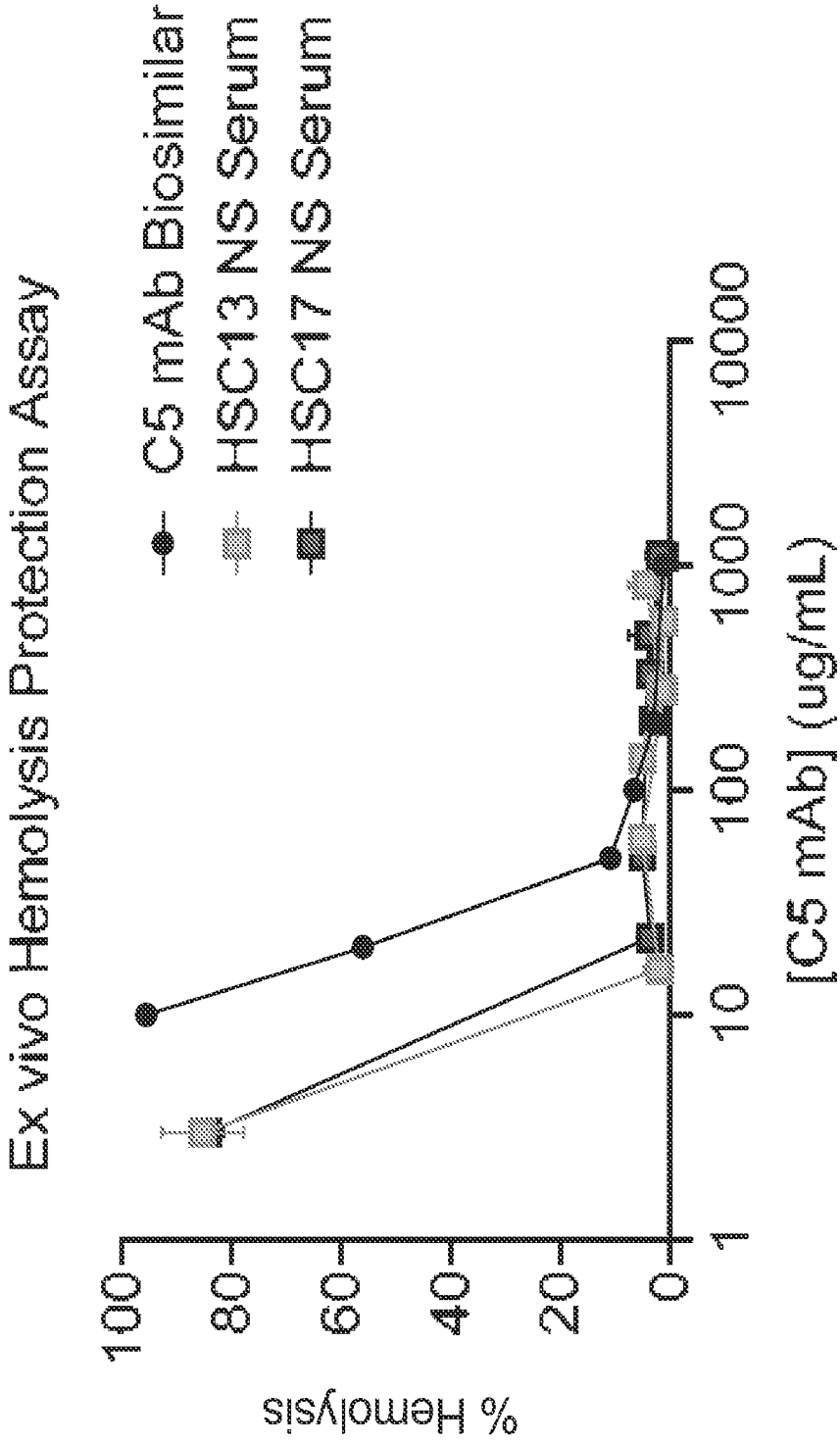
Ex vivo Hemolysis Protection Assay
C5 mAb Biosimilar
HSC13 NS Serum
HSC17 NS Serum
% Hemolysis
100
80
60
40
20
0
1
10
100
1000
10000
[C5 mAb] (ug/mL)

FIG. 5

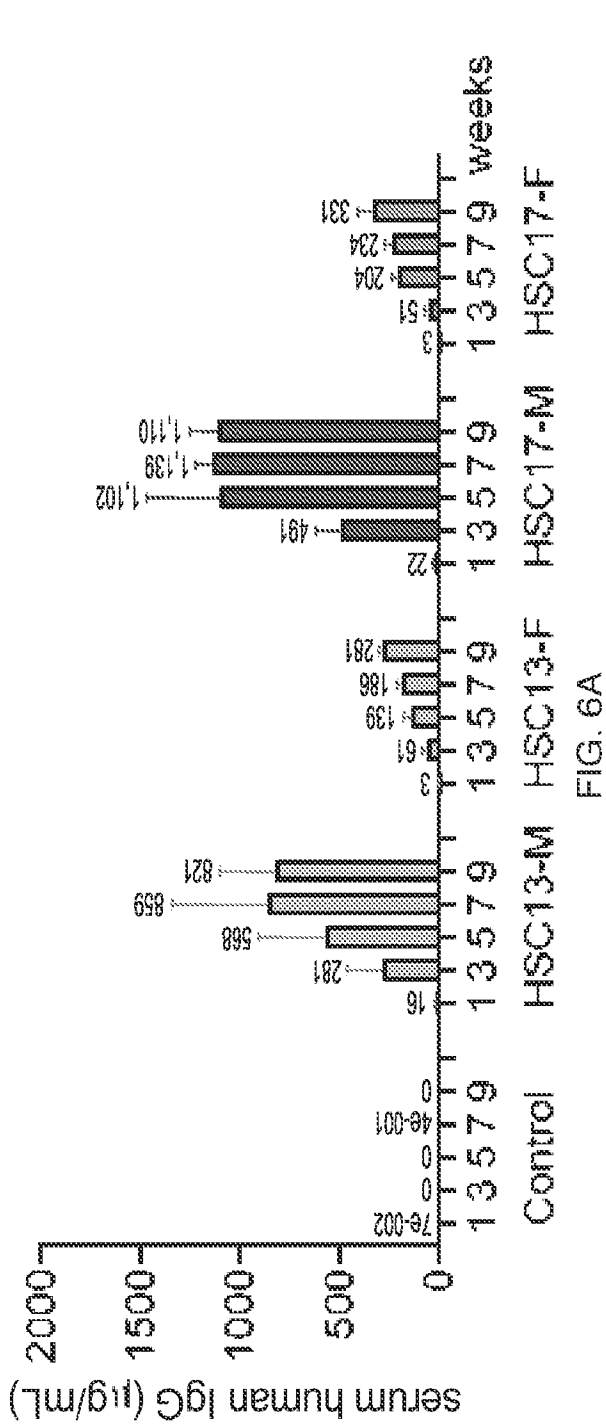
serum human IgG (µg/mL)
2000
1500
1000
500
0
76-002
0
0
46-001
0
16
281
568
859
821
3
61
139
186
281
22
491
1,102
1,139
1,110
3
51
204
234
331
1 3 5 7 9 1 3 5 7 9 1 3 5 7 9 weeks
Control HSC13-M HSC13-F HSC17-M HSC17-F

FIG. 6A

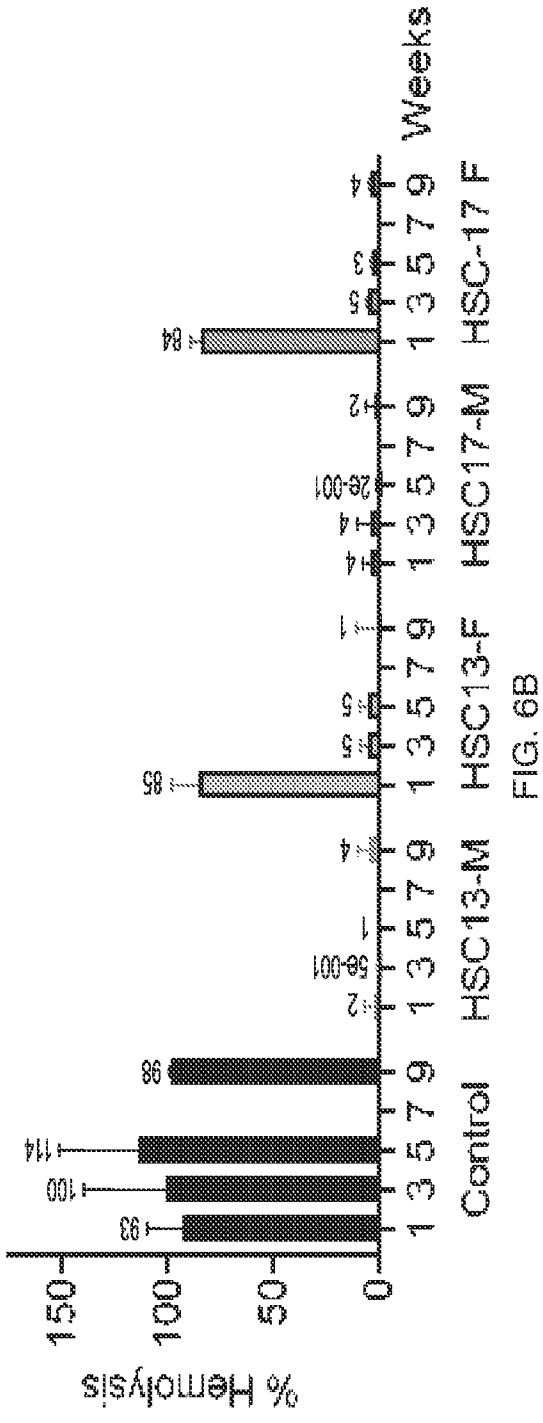
% Hemolysis
150
100
50
0
93
100
114
98
2
56-001
1
4
85
5
5
1
14
4
26-001
2
84
5
3
4
1 3 5 7 9 1 3 5 7 9 1 3 5 7 9 Weeks
Control HSC13-M HSC13-F HSC17-M HSC-17 F

FIG. 6B

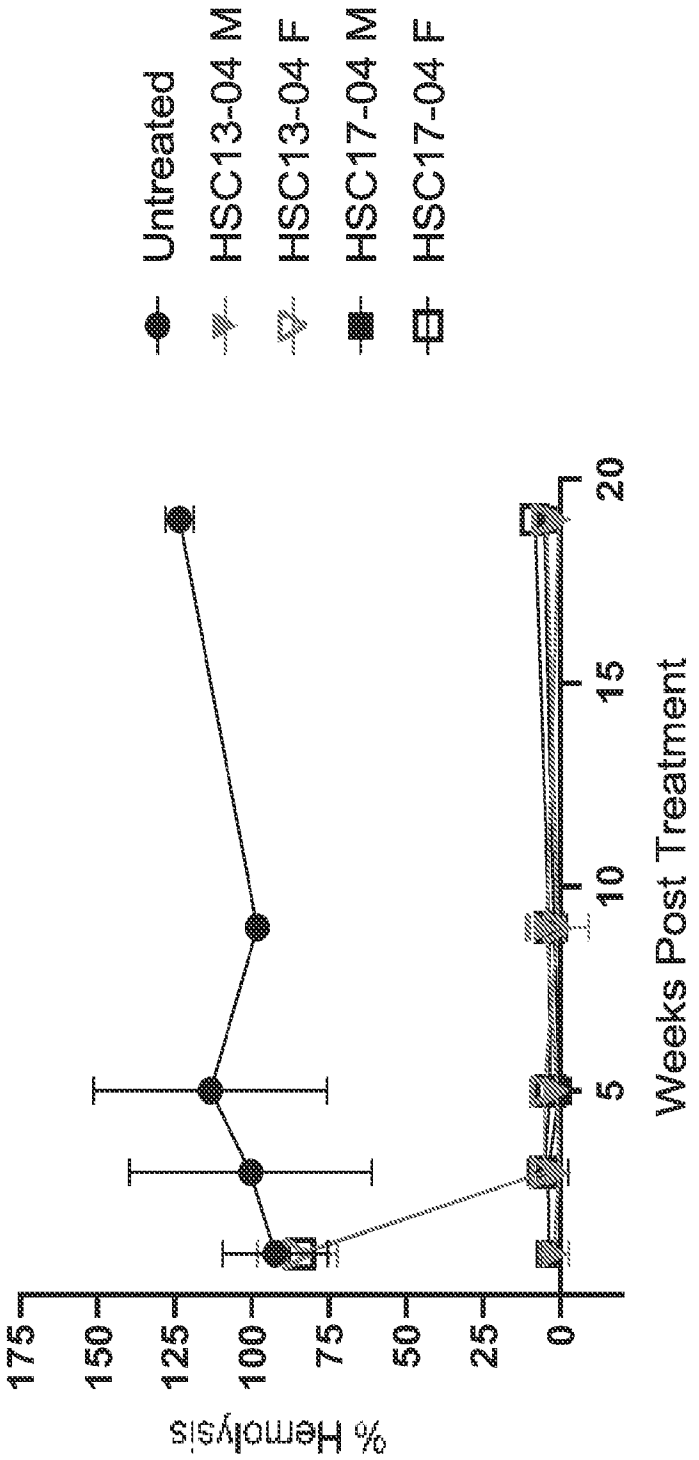
Untreated
HSC13-04 M
HSC13-04 F
HSC17-04 M
HSC17-04 F
% Hemolysis
175
150
125
100
75
50
25
0
5
10
15
20
Weeks Post Treatment

FIG. 6C

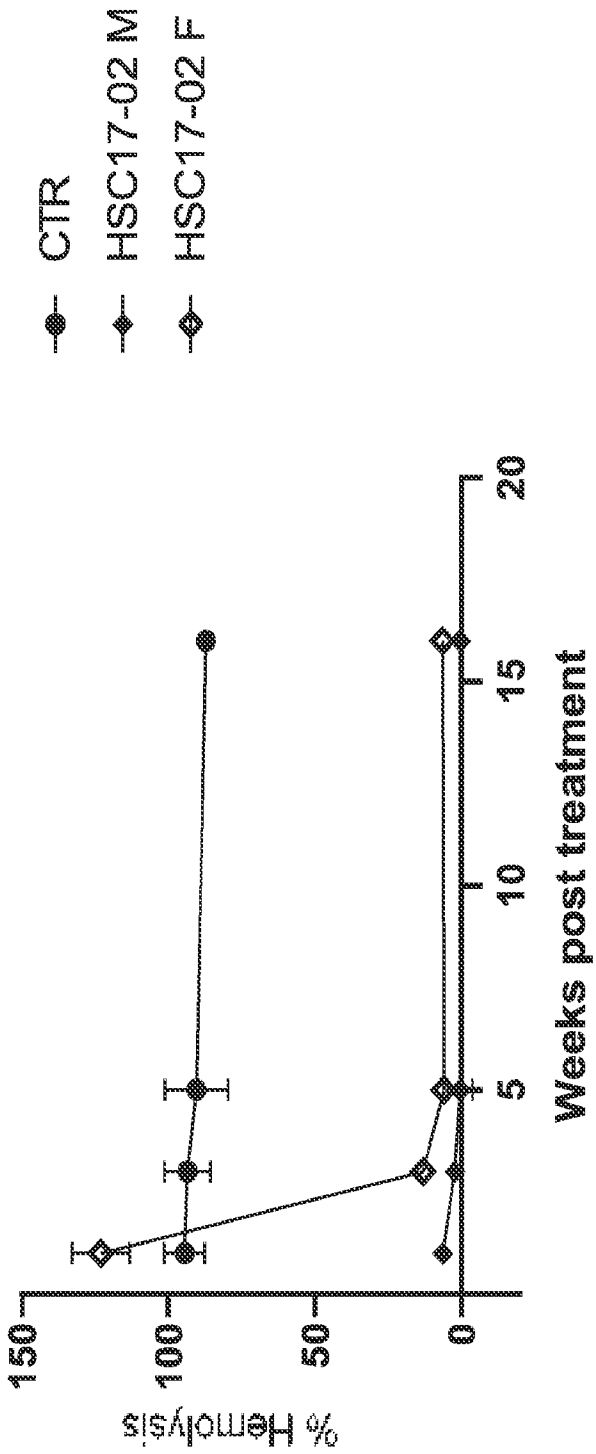
CTR
HSC17-02 M
HSC17-02 F
% Hemolysis
150
100
50
0
0
5
10
15
20
Weeks post treatment

FIG. 6D

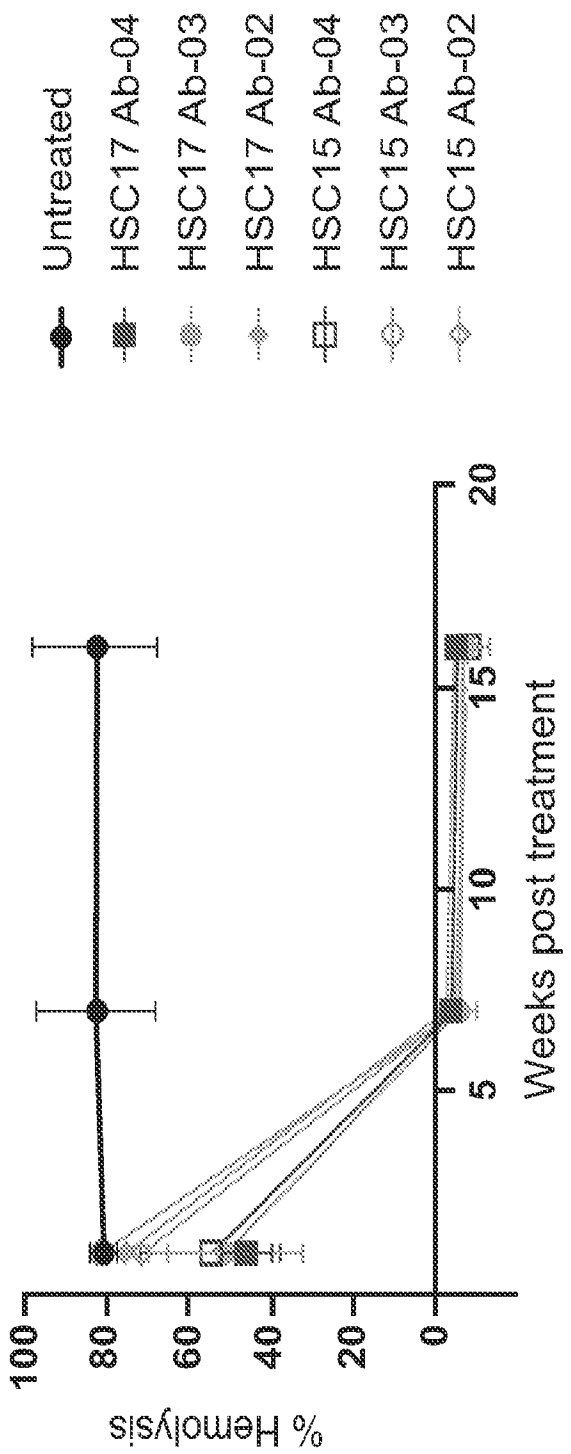
Untreated
HSC17 Ab-04
HSC17 Ab-03
HSC17 Ab-02
HSC15 Ab-04
HSC15 Ab-03
HSC15 Ab-02
% Hemolysis
100
80
60
40
20
0
5
10
15
20
Weeks post treatment

FIG. 6E

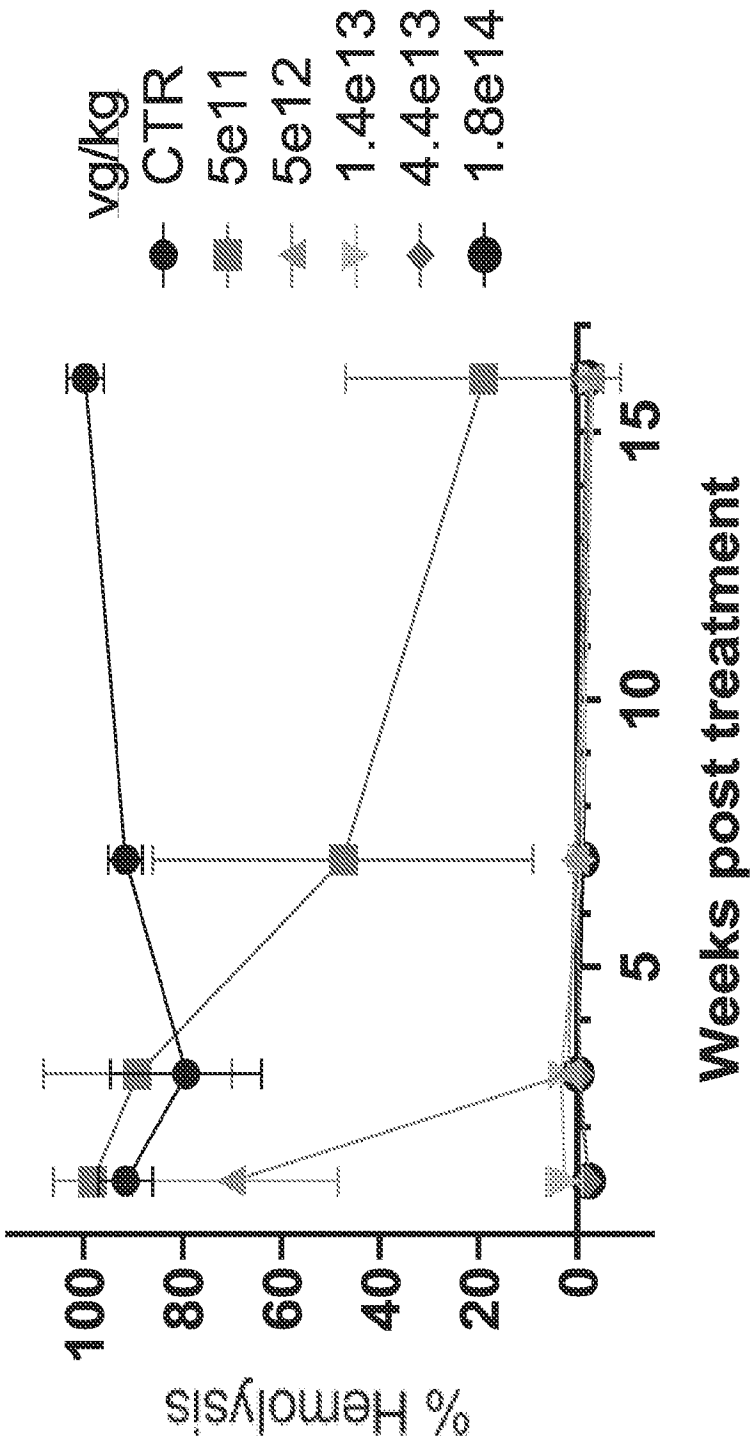
vg/kg
CTR
5e11
5e12
1.4e13
4.4e13
1.8e14
% Hemolysis
100
80
60
40
20
0
5
10
15
Weeks post treatment

FIG. 6F

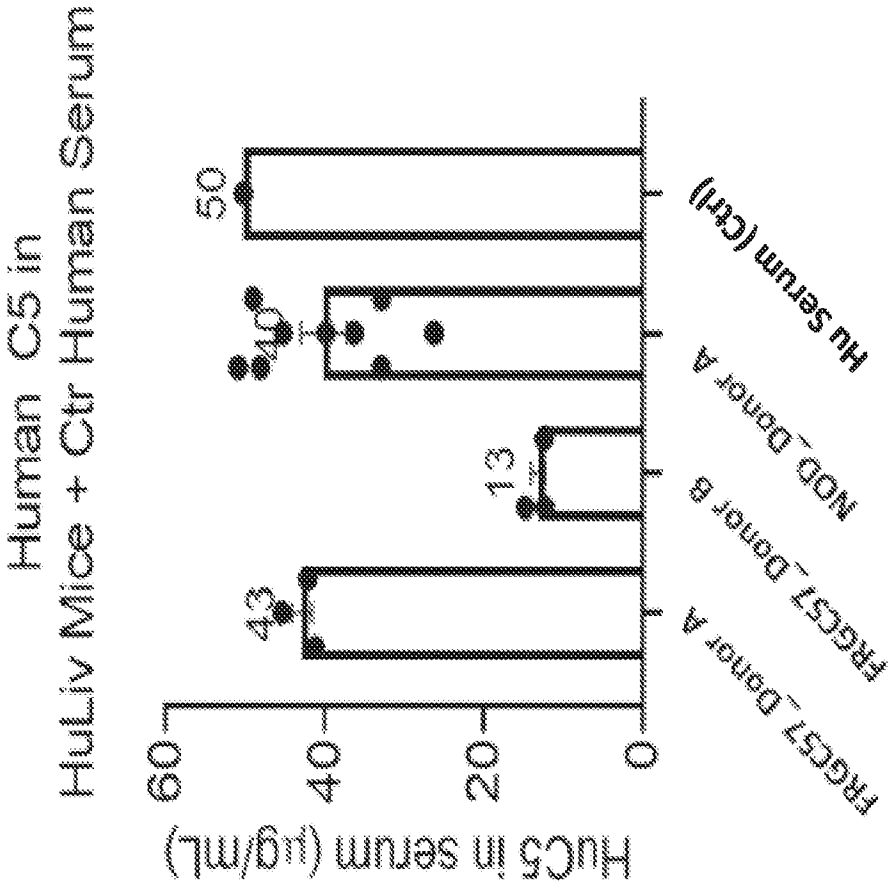
Human C5 in
HuLiv Mice + Ctr Human Serum
HuC5 in serum (µg/mL)
0
20
40
60
43
13
40
50
FRGCS7_Donor A
FRGCS7_Donor B
NOD_Donor A
Hu Serum (Ctrl)

FIG. 7A

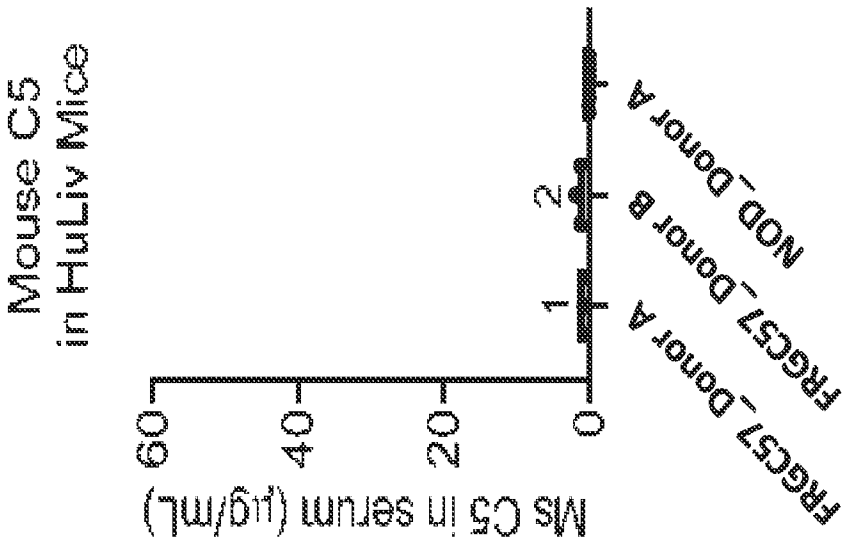
Mouse C5
in HuLiv Mice
Ms C5 in serum (µg/mL)
0
20
40
60
1
2
FRGCS7_Donor A
FRGCS7_Donor B
NOD_Donor A

FIG. 7B

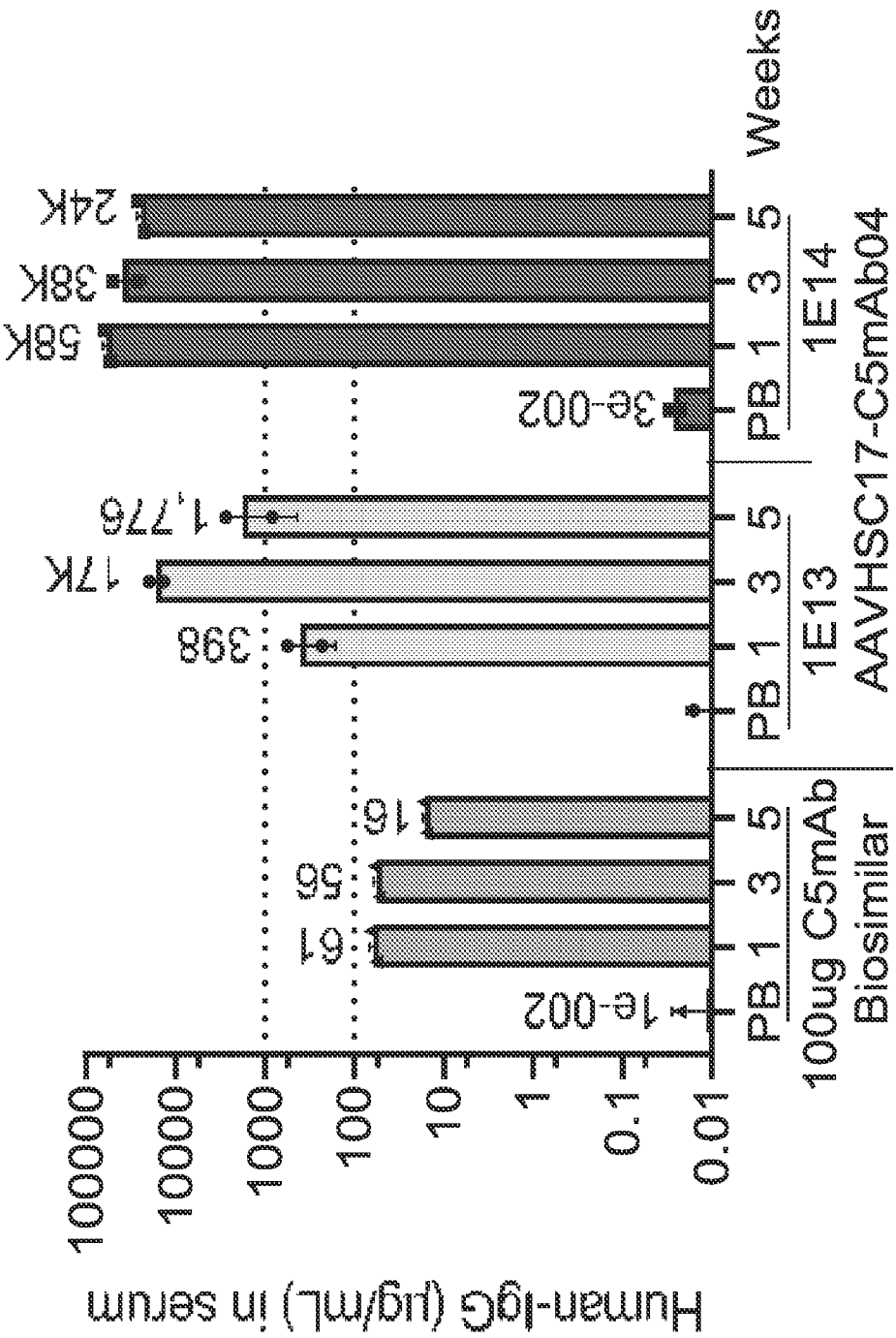
Human-IgG (µg/mL) in serum
100000
10000
1000
100
10
1
0.1
0.01
100ug C5mAb Biosimilar
1E13 AAVHSC17-C5mAb04
1E14
PB 1 3 5
PB 1 3 5
PB 1 3 5
Weeks
1e-002
61
56
16
398
17K
1,776
3e-002
58K
38K
24K

FIG. 8A

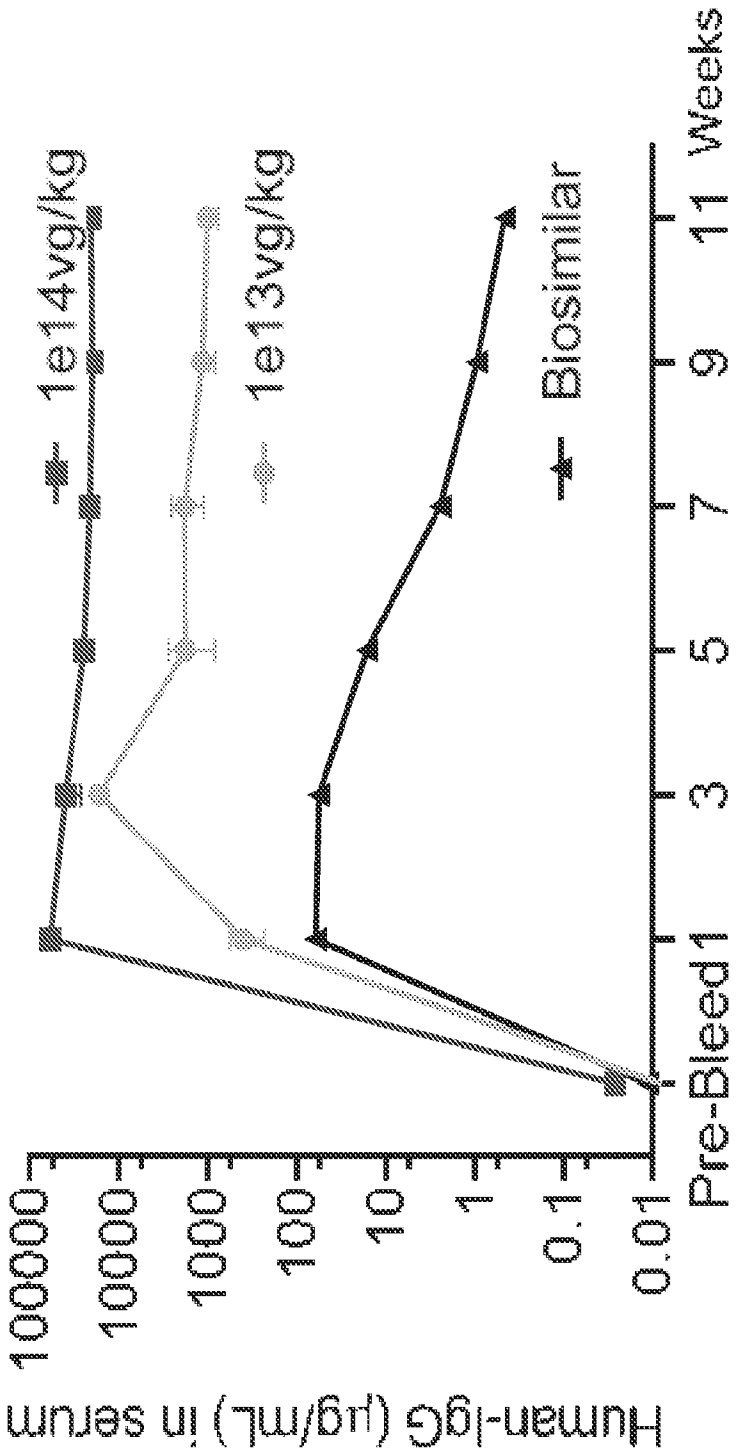
Human-IgG (µg/mL) in serum
100000
10000
1000
100
10
1
0.1
0.01
Pre-Bleed1
3
5
7
9
11 Weeks
1e14vg/kg
1e13vg/kg
Biosimilar

FIG. 8B

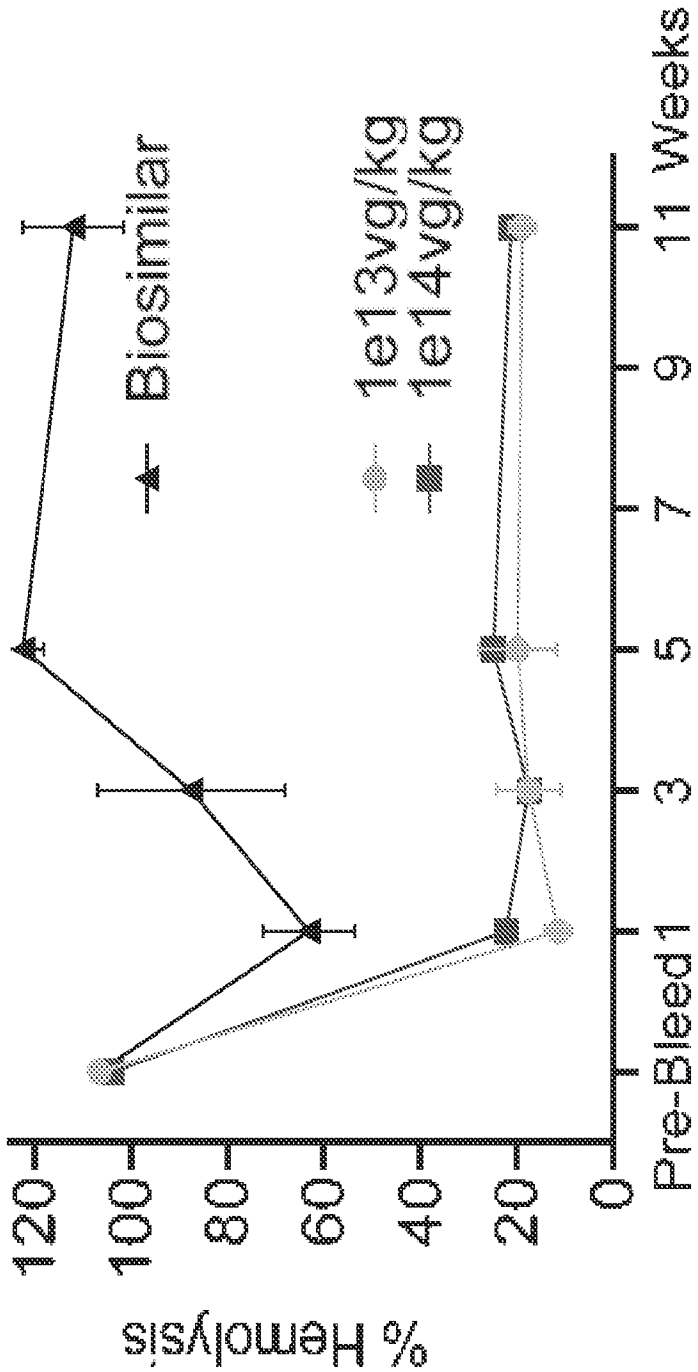
% Hemolysis
120
100
80
60
40
20
0
Pre-Bleed
1
3
5
7
9
11 Weeks
Biosimilar
1e13vg/kg
1e14vg/kg

FIG. 8C

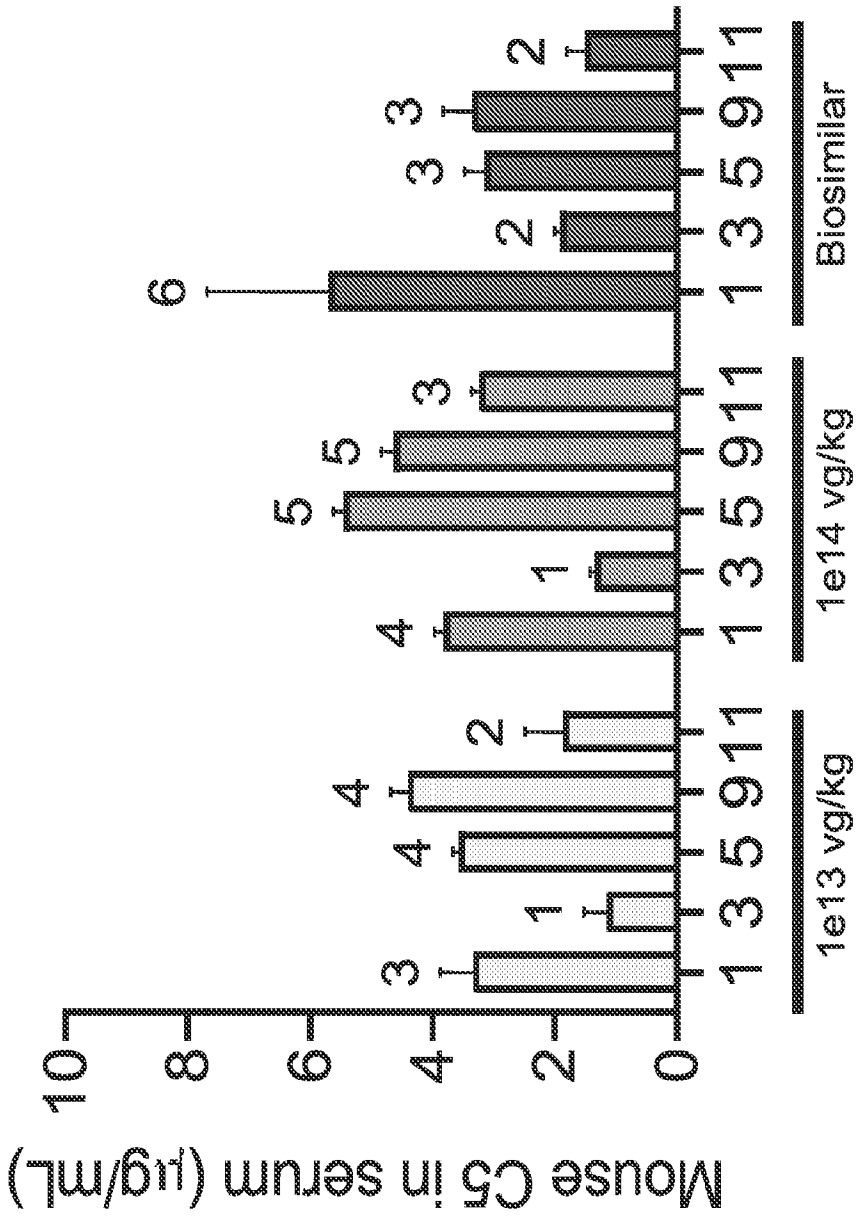
Mouse C5 in serum (μg/mL)
0
2
4
6
8
10
1 3 5 9 11
1e13 vg/kg
1 3 5 9 11
1e14 vg/kg
1 3 5 9 11
Biosimilar

FIG. 8D

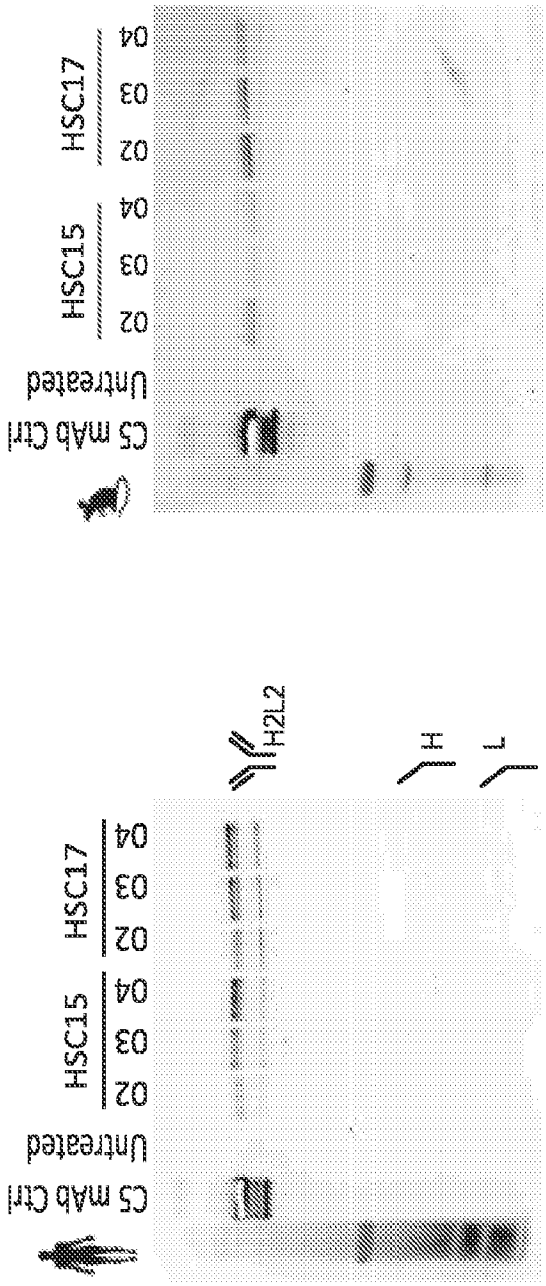
C5 mAb Ctrl
Untreated
HSC15
02
03
04
HSC17
02
03
04
H2L2
H
L
Western Blot

FIG. 9A

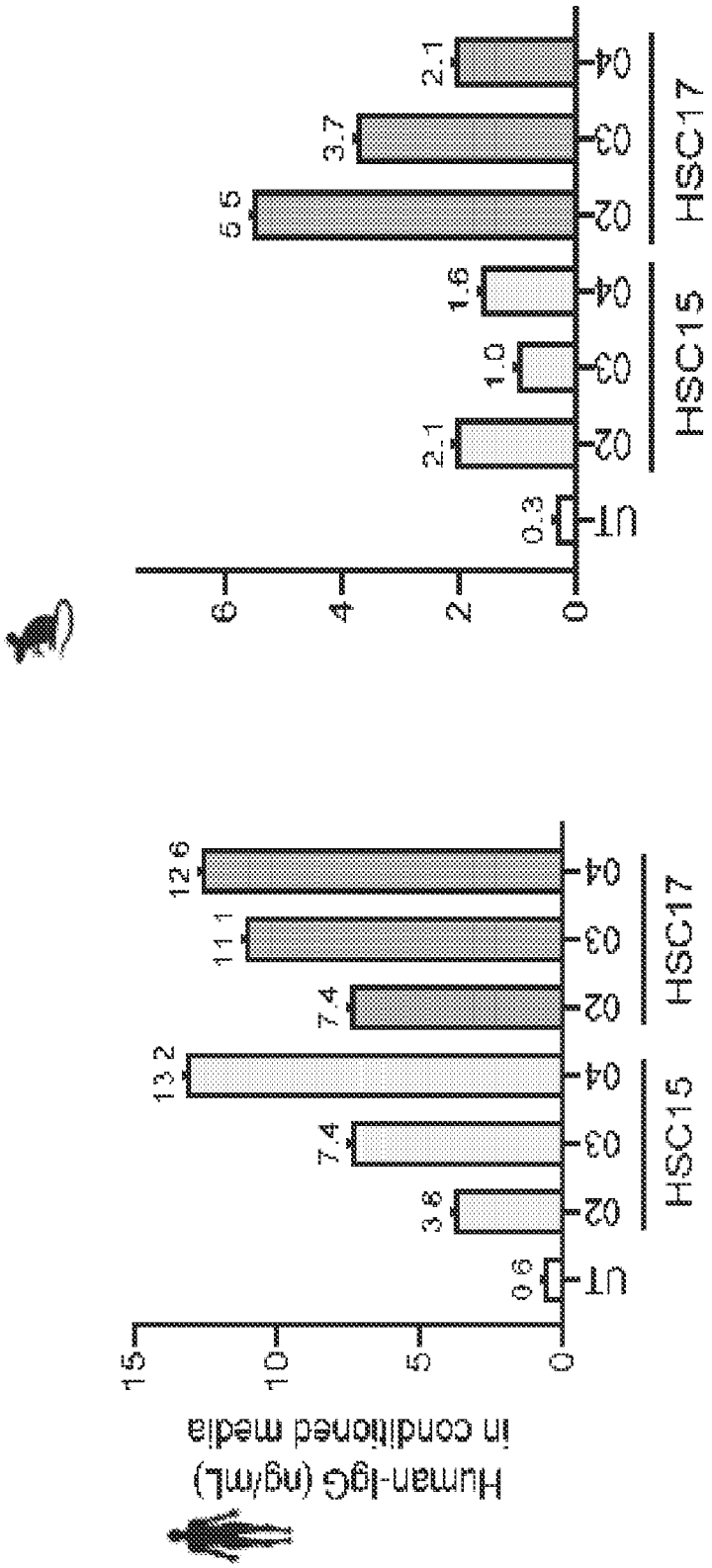
Human-IgG (ng/mL)
in conditioned media
15
10
5
0
UT
02
03
04
HSC15
HSC17
0.6
3.8
7.4
13.2
7.4
11.1
12.6
Human IgG ELISA
0
2
4
6
UT
02
03
04
HSC15
HSC17
0.3
2.1
1.0
1.6
5.5
3.7
2.1

FIG. 9B

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/71400

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/86, A61K 48/00, A61P 7/02 (2022.01)

CPC - C12N 15/8645, C12N 2710/10343, A61P 7/06, A61K 2039/505, C12N 2710/10043

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 2012/0027798 A1 (CLARK et al.) 2 February 2012 (02.02.2012); Abstract; para [0018], [0019], [0020], [0036], [0038], [0045], [0047], [0061] | 1 |
| Y | | 2-3, 49-51 |
| Y | US 2017/0216456 A1 (THE SYDNEY CHILDREN'S HOSPITALS NETWORK (RANDWICK AND WESTMEAD (INCORPORATING THE ROYAL ALEXANDRA; CHILDREN'S MEDICAL RESEARCH INSTITUTE) 3 August 2017 (03.08.2017) Abstract, para [0023], SEQ ID NO: 25 | 2-3, 50-51 |
| Y | US 2017/0088856 A1 (LELAND STANFORD JUNIOR UNIVERSITY) 30 March 2017 (30.03.2017) para [0052], [0059] | 49-51 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 25 January 2022 | FEB 14 2022 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300 | Authorized officer<br>Kari Rodriquez<br>Telephone No. PCT Helpdesk: 571-272-4300 |

Form PCT/ISA/210 (second sheet) (July 2019)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/71400

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/71400

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-48, 52-54, 58-128 and 130-145
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

*****Continued in Supplemental Box*****

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3 and 49-51, limited to SEQ ID NO: 25

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continuation of Box No. III (Observations where unity of invention is lacking);

Group I+: Claims 1-3, 49-51, 55-57 and 129 directed to a recombinant adeno-associated virus (rAAV) genome comprising first and second expression cassettes, each cassette comprising (5'-3'), a first or second liver-specific transcriptional regulatory element, a first or second signal sequence, and a first or second coding sequence for a heavy and a light chain (antibody polypeptides), and a first or second polyadenylation sequence; optionally wherein the first and second cassettes are separated by a ribosomal skipping peptide sequence; wherein expression of the first and second coding sequences produces an antibody. The rAAV genome will be searched to the extent that the first and/or second transcriptional regulatory elements comprise a promoter element comprising a nucleic acid sequence at least 90% identical to SEQ ID NO: 25. It is believed that claims 1-3 and 49-51 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass said rAAV genome. Additional rAAV genomes will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected rAAV genomes. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a rAAV genome comprising first and second expression cassettes transcriptional regulatory elements having promoter elements comprising nucleic acid sequences at least 90% identical to SEQ ID NOs: 25 and 27, respectively (claims 1-3 and 49-51). Another exemplary election would be a rAAV genome comprising first and second expression cassettes, separated by a ribosomal skipping peptide sequence; wherein the transcriptional regulatory elements comprise the nucleotide sequence set forth in SEQ ID NO: 67 (claims 49-50 and 55-57). Another exemplary election would be a rAAV polynucleotide comprising the nucleic acid sequence set forth in SEQ ID NO: 86 [i.e. Anti-CS antibody vector C5Ab04, as shown in FIG. 1 and, as shown in Table 1, specific sequences for transcriptional regulatory elements are SEQ ID NOs: 43 and 50, also see para [00210], instant specification] (Claim 129).

The inventions listed as Group I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

No technical features are shared between the transcriptional regulatory element nucleotide sequences and/or the rAAV genome nucleic acid sequences of Group I+, accordingly, these groups lack unity a priori.

*Additionally, even if the inventions listed as Group I+ were considered to share technical features, these shared technical features are* previously disclosed by the prior art, as further discussed below.

Common Technical Features

The inventions of Groups I+ share the technical feature of rAAV genomes comprising sequence elements for the expression of an antibody, including liver-specific transcriptional regulatory element nucleotide sequences

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by US 2012/0027798 A1 to Clark et al. (hereinafter 'Clark').

Clark teaches rAAV genomes comprising sequence elements for the expression of an antibody, including liver-specific transcriptional regulatory element nucleotide sequences (abstract "The present invention relates generally to the use of recombinant adeno-associated viruses (rAAV) for gene delivery and more specifically to the use of rAAV to deliver antibody genes to target cells in mammals.", para [0018] "the invention provides rAAV genomes. The rAAV genomes comprise AAV ITRs flanking a gene cassette of DNA encoding one or more antibody polypeptides operatively linked to transcriptional control DNA, specifically promoter DNA and polyadenylation signal sequence DNA, functional in target cells.", para [0019] "In particular, the invention contemplates a dual promoter gene cassette which encodes light and heavy chain polypeptides. In one embodiment, the gene cassette contains the following: (1) two constitutive promoters that are active in the cell that will be transduced, (2) several unique restriction enzyme sites to allow for the rapid replacement of promotor elements or heavy and light chain coding sequences, (3) unique restriction sites that facilitate in-frame antibody gene cloning, (4) a strong transcriptional termination site 3' to the first expression cassette to reduce possible promoter interference and (5) polynucleotide sequences encoding both the heavy and light chain of a monoclonal antibody of interest each inserted under the transcriptional control of one of the two promoters.", para [0036] "Transduction of cells with rAAV of the invention results in sustained expression of antibody polypeptide(s). The present invention thus provides methods of delivering rAAV which express antibody polypeptides to an animal, preferably a human being. These methods include transducing tissues (including but not limited to muscle, liver and brain) with one or more rAAV of the present invention. Transduction may be carried out with gene cassettes comprising tissue specific control elements.").

As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the inventions.

Group I+ therefore lacks unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

*Item 4, continued: claims 4-48, 52-54, 58-128 and 130-145 are not drafted in accordance with the second and third sentences of Rule 6.4(a) regarding multiply dependent claims.