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(54) Title: COMPOSITION AND METHOD FOR TREATING COMPLEMENT-MEDIATED DISEASE

(57) Abstract: A recombinant vector having an expression cassette comprising a modified human factor H (hfH) gene is provided, wherein said hfH gene encodes a hfH protein variant comprising SCR1-4, 19-20, and one or more of SCR7, SCR17 and/or SCR18. Also provided are pharmaceutical compositions containing this vector and uses therefor in treating AMD and/or other complement associated diseases.

COMPOSITION AND METHOD FOR TREATING COMPLEMENT-MEDIATED DISEASE

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under Grant No. AI085596 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

The complement system is a part of innate immunity that plays a key role in host defense. Complement can be activated by three different pathways, the classical, alternative and lectin pathways. Among them, the alternative pathway is unique in that it not only represents an independent pathway by which complement is activated by the "tick-over" mechanism, but also it amplifies complement activation initiated by the other two pathways. The alternative pathway requires the participation of C3, factor B (fB), factor D (fD) and properdin (fP). All pathways converge at the C3 activation step from where the alternative pathway amplification loop comes into play. Regardless of which pathway complement activation occurs, activated complement produces three types of effector functions: opsonization of targets with C3b/iC3b/C3d to facilitate phagocytosis and clearance, production of pro-inflammatory mediators C3a and C5a, and direct cellular attack by the terminal complement activation effector C5b-9, also known as membrane attack complex (MAC). Through activation of complement receptors (CRs) such as CR2 on B cells and follicular dendritic cells, and anaphylatoxin receptors C3a receptor (C3aR) and C5a receptor (C5aR) on leuckocytes such as macrophages and monocytes, complement also interacts with and cross-regulates the adaptive immune systems and thus plays a modulatory role in B and T cell immunology.

A number of human diseases are caused by complement dysregulation, resulting in complement-mediated autologous tissue injury. The complement dysregulation may arise from mutations, either somatic or germline, in complement regulator or regulator-related genes such that these regulators no longer function normally. Examples of this

category include mutations in hematopoietic stem cells of the PIG-A gene that encodes for a key enzyme in the GPI anchor biosynthesis and such mutations result in the lack of expression of DAF and CD59 on blood cells of paroysmal nocturnal hemoglobinuria (PNH) patients. As a result, PNH patient's red blood cells and platelets are not protected from complement attack and they develop intravascular hemolysis and platelet activation, leading to anemia and thrombotic attacks. A second example is mutation in the membrane regulator MCP or fluid phase regulators fH or fI which render over-activation of the alternative pathway of complement in the kidney, leading to the pathogenesis of C3 glomerulopathy or atypical hemolytic uremic syndrome (aHUS). In addition to such rare and high penetrant mutations leading to absence of expression or dysfunction of DAF, CD59, fH, fI and MCP, there are single nucleotide polymorphisms (SNP) in fH that are more prevalent and less penetrant but nevertheless have been identified to contribute to disease pathogenesis via a complement-mediated mechanism. A very well characterized example is the strong association of Y420H polymorphism in fH with age-related macular degeneration (AMD). Thus, complement regulator dysfunction or sequence variation may lead to common as well as rare human diseases.

Complement dysregulation may arise not only from regulatory mutation/polymorphism but also from mutations in genes that encode the critical components of the alternative pathway, namely C3 and fB, as well as by the presence of autoantibodies against regulators or complement proteins such as fH, C3 or fB. It is now understood that certain mutations in C3 or fB will result in proteins which, when activated, form an unusually stable alternative pathway C3 convertase C3bBb that is resistant to regulation by the regulatory proteins, which in turn can lead to complement dysregulation and over-activation. In the case of autoantibodies against complement regulators, they often mimic mutations in genes encoding such proteins with the result being reduced functional potency of such proteins in the fluid phase or on the cell surface. Separately, autoantibodies against C3b called C3 nephritic factors (C3nef) are capable of binding and stabilizing the alternative pathway C3 convertase C3bBb, thus achieving the same effect of prolonging the half-life and activity of the convertase as that produced by C3 or fB gene mutations. Overall, there are common and rare human diseases that are caused by excessive complement activation resulting from dysregulation of the

complement activation cascade. The underlying mechanism of complement dysregulation are variable, some are due to gene mutations and others to autoantibodies, and the mutated genes or targets of autoantibodies could be regulatory proteins or components of the alternative pathway.

Current therapeutic approaches are focused on the development of reagents such as mAbs, peptides or other small molecules that bind and block specific alternative pathway or terminal pathway complement components. A clinically validated example is Eculizumab, a humanized mAb against complement C5 which has been approved for the treatment of PNH and aHUS. Other approaches that have been described include mAbs against fB, fD, or fP, and a cyclic peptide that binds and inhibits C3. The limitation of these approaches is that they require repeated and inconvenient IV dosing of patients. Further, since they block the alternative pathway or terminal pathway, they run the risk of compromising host defense. Indeed, patients on Eculizumab therapy have to be vaccinated against bacteria strains that cause lethal meningitis and these patients are also put on prophylactic antibiotic therapy before being treated with the approved mAb drug.

In other approaches, recombinant regulatory proteins such as soluble DAF, CR1, CRIg and proteins comprising minimal domains of fH (N-terminal short consensus repeat [SCR] 1-5 and C-terminal SCR 19-20) or fusion proteins between fH and CR2 (TT30) have been tested. See, e.g., US Patent Publication No. US2013/0296255; US Patent Publication No. 2008/0221011. However, large scale heterologous expression of such proteins as therapeutic drugs requires significant effort, and animal studies have shown their *in vivo* clearance rate after administration to be fast (Nichols EM, Barbour TD, Pappworth IY, Wong EK, Palmer JM, Sheerin NS, Pickering MC, Marchbank KJ. Kidney Int. 2015 Jul 29. doi: 10.1038/ki.2015.233.; Fridkis-Hareli M, Storek M, Mazsaroff I, Risitano AM, Lundberg AS, Horvath CJ, Holers VM, Blood. 2011 Oct 27;118(17):4705-13. doi: 10.1182/blood-2011-06-359646. Epub 2011 Aug 22.), making such therapeutic strategies cumbersome and less practical as multiple and frequent administrations of such protein drugs would be required.

A need remains in the art for compositions useful for treating complement-mediated diseases with greater and longer-lasting efficacy.

SUMMARY OF THE INVENTION

In one aspect, the invention provides a recombinant vector having packaged therein an expression cassette comprising an engineered human complement regulator factor H (fH) gene operably linked to expression control sequences which direct expression thereof, wherein said hfH gene encodes a soluble hfH protein variant that retains complement regulatory function, wherein said fH variant comprises short consensus repeat (SCR) 1, 2, 3, 4, 19 and 20 and at least one of SCR7, SCR17 and/ SCR18, wherein following administration of the vector to a subject and expression, detectable plasma levels of the hfH variant are present in the subject for at least a week.

In another aspect, the invention provides a recombinant AAV vector having packaged therein an expression cassette comprising an engineered human complement regulator factor H (fH) gene operably linked to expression control sequences which direct expression thereof, wherein said hfH gene encodes a soluble hfH protein variant that retains complement regulatory function, wherein said fH variant comprises short consensus repeat (SCR) 1, 2, 3, 4, 19 and 20, wherein following administration of the vector to a subject and expression, detectable therapeutically useful plasma levels of the hfH variant are present in the subject for at least about a month.

In a further aspect, a pharmaceutical composition is provided which comprises a carrier and/or excipient and a recombinant vector as described herein which expresses an fH variant.

In yet another aspect, a method is provided for treating a complement related disorder by delivering to the subject a vector as described herein. The complement related disorder may be, among others, membranoproliferative glomerulonephritis, atypical hemolytic uremic syndrome (aHUS), age related macular degeneration (AMD), microangiopathic haemolytic anemia, thrombocytopenia, acute renal failure, paroxysmal nocturnal hemoglobinuria (PNH), schizophrenia, ischemic stroke, and/or bacterial infections caused by recruitment of bacterial pathogens.

In a further aspect, use of a recombinant vector for treating AMD is provided. In another aspect, use of a rAAV vector for treating PNH, aHUS, or another complement associated disorder is described.

In another aspect, an engineered hfH variant is provided which comprises a leader sequence and human complement receptor SCRs consisting of: (a) SCR1-4, 7, and 19-20; (b) SCR1-4, 6, 7, and 19-20; (c) SCR1-4, 7, 8, and 19-20; ~~or~~ (d) SCR1-4, 6, 7, 8, and 19-20; (e) SCR1-4, 17, and 19-20; (f) SCR1-4, and 18-20; (g) SCR1-4, and 17-20. Other embodiments include, e.g., SCR1-4, 7, and 18-20; SCR1-4, 6, 7, and 18-20; SCR1-4, 7, 8, and 18-20; or SCR1-4, 6, 7, 8, and 18-20, SCR1-4, 7, and 17-20; SCR1-4, 6, 7, and 17-20; SCR1-4, 7, 8, and 17-20; or SCR1-4, 6, 7, 8, and 17-20. Optionally, at least one glycosylation site is engineered into at least one of the SCRs. In another aspect, one of the engineered hfH variants is pegylated.

In still another aspect, a pharmaceutical composition comprising at least one type of the engineered hfH variant, a carrier and/or an excipient is provided. Such a composition may be used on its own, or in combination with another therapy, particularly, e.g., the vector therapy described herein.

Other aspects and advantages of the invention will be readily apparent from the following detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG 1A provides a schematic of the domain structure of mature human factor H protein.

FIGs 1B-1E provide the nucleic acid and amino acid sequences of the leader peptide and identifies the locations of the 20 Short Consensus Repeat (SCR) domains used in generating the fH variants illustrated in the examples below. SEQ ID NO:1 provides the nucleic acid sequences; SEQ ID NO: 2 provides the amino acid sequence of the signal peptide. The amino acid sequences of the SCR1-20 are provided in SEQ ID NO: 3 (SCR1), 5 (SCR2), 7 (SCR3), 9 (SCR4), 11 (SCR5), 13 (SCR6), 14 (SCR7), 16 (SCR8), 17 (SCR9), 19 (SCR10), 21 (SCR11), 23 (SCR12), 25(SCR13), 27 (SCR14), 29 (SCR15), 31 (SCR16), 33 (SCR17), 35 (SCR18), 37 (SCR19), and 38 (SCR20) respectively. The locations of these domains in the fH isoform 1 are based on the convention described in C. Estaller et al, Eur J Immunol. 1991 Mar; 21(3):799-802. The amino acids sequences between the defined SCRs are linker sequences that afford fH flexibility [SEQ ID NO: 4, 6, 8, 10, 12, 15, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36],

respectively]. The linker between SCR19 and SCR20 is only three amino acids (Leu-His-Pro), and thus not generated by the features in the Sequence Listing.

FIG 2A provides a schematic domain structure of human factor H variant containing SCR1-4, 6-8, and 19-20.

FIGs 2B–2C provide the nucleic acid [nt 53 - 1804 of SEQ ID NO: 41] and amino acid sequences [SEQ ID NO: 42] of the leader peptide and 9 short consensus repeat (SCR) domains of the fH variant SCR1-4, 6-8 and 19-20.

FIGs 3A-3B are the complete cDNA [nt 53-1804 of SEQ ID NO: 41] and 5'- [nt 1-52 of SEQ ID NO:41] and 3'- UTR [nt 1805 - 2068 of SEQ ID NO: 41] sequences of the human factor H truncation construct containing the leader peptide and SCR1-4, 6-8, and 19-20 (hfH1-4.678.19-20).

FIG 4 is the amino acid sequence of the factor H truncation construct containing the leader peptide (underlined) and SCR1-4, 6-8, 19-20 (hfH1-4.678.19-20) [SEQ ID NO: 42].

FIGs 5A and 5B are gels which provide confirmation of protein expression and stability of hfH1-4.678.19-20. The cDNA sequence shown in Fig 2 of human fH truncation variant containing SCR1-4, 6-8, and 19-20 [SEQ ID NO: 41] was cloned into eukaryotic expression vectors which were then used to transfect HEK cells. Cell culture supernatant was used for western blot analysis to detect truncated fH protein expression. Panel A: Lane 1, untransfected HEK cells; Lane 2 and 3, HEK cells transfected with a pCMV Sport6 vector containing the fH truncation variant cDNA; Lane 4-6, HEK cells transfected with a pCBARBG vector containing the fH variant cDNA. The pCBARBG vector contains the same 5' and 3' regulatory elements as the pAAV vector construct shown in Fig 4. Panel B: Lane 1, untransfected HEK cells; Lane 2, HEK cells transfected with a pCBARG vector containing the truncated fH variant cDNA as a control; Lane 3, HEK cells transfected with the AAV8 plasmid containing the truncated fH variant cDNA.

FIG 6 is an SDS-gel which shows purification of recombinant hfH1-4.678.19-20. SDS-gel analysis was performed via Coomasie blue staining of human fH truncation variant containing SCR1-4, 6-8, and 19-20 that was expressed by transfecting HEK cells using the pCBARBG vector. The recombinant fH truncation protein was purified from

the supernatant by passing through an affinity column that was prepared using a mAb against human factor H (clone OX-23) that recognizes an epitope in SCR2-3. Size and location of protein molecular weight markers are shown on the left side.

FIG 7 is a gel showing recombinant hfH1-4.678.19-20 retains complement regulating activity (cofactor activity). The human fH truncation variant containing SCR1-4, 6-8, and 19-20 was tested for cofactor activity for factor I-mediated C3b cleavage. For this assay, human C3b was mixed with factor I in the presence (Lane 1-6) or absence (Lane 7) of full-length fH (hfH) or the truncated fH variant (hfH1-4.678.19-20). The reaction mixture was incubated and then analyzed by SDS-PAGE and western blot analysis. Cofactor activity is indicated by the appearance of the iC3b α-chain fragment.

FIG 8 is a line graph showing that recombinant hfH1-4.678.19-20 (square, top line) has strong heparin-binding activity. The human fH truncation variant containing SCR1-4, 6-8, and 19-20 retains heparin-binding activity. Its heparin-binding activity is dose-dependent, and when compared with full-length human fH (diamond, lower line) on a μg/ml basis, it showed higher activity. Heparin-binding activity was assessed by ELISA using plate-coated heparin, overlay of a full-length or truncated fH protein solution and, after washing, detection of bound fH or truncated fH by the mAb OX-23 (against an epitope in SCR2-3).

FIG 9 is a line graph showing recombinant hfH1-4.678.19-20 (square, top line) has strong C3b-binding activity. The human fH truncation variant containing SCR1-4, 6-8, and 19-20 retains C3b-binding activity. Its C3b-binding activity is dose-dependent, and when compared with full-length human fH (diamond, bottom line) on a μg/mL basis, it showed higher activity. C3b-binding activity was assessed by ELISA using plate-coated C3b, overlay of a full-length or truncated fH protein solution and, after washing, detection of bound fH or truncated fH by the mAb OX-23 (against an epitope in SCR2-3).

FIG 10 is a line graph showing ELISA detection of hfH1-4.678.19-20 in the blood of 3 different fH mutant mice ($fH^{m/m}$; F1, F2, F20) one week after AAV8-mediated fH gene therapy. The $fH^{m/m}$ mouse is a strain of fH mutant mice that carry premature stop codons at the beginning of SCR19. These mice produce trace amount of truncated fH

(lacking SCR19-20) and has uncontrolled fluid phase alternative pathway complement activation and consumption (secondary C3 and fB deficiency). Mice were infected by retro-orbital I.V. with an AAV8 virus containing hfH1-4.678.19-20 (3×10^{11} gene copies/mouse) and after one week, blood samples were collected and processed for human fH protein detection. For ELISA assay, the mAb OX-23 was used as a capture antibody (recognizing an epitope in human fH SCR2-3) and biotinylated mAb L20/3 was used as a detection antibody (recognizing human fH SCR19). As shown in the figure, there is no hfH1-4.678.19-20 in the blood of 3 $fH^{m/m}$ mice (F1, F2, F20) before AAV-hfH1-4.678.19-20 treatment (Pre), but hfH1-4.678.19-20 was detected one week (1W) after treatment.

FIG 11 is western blot analysis demonstrating that AAV8-mediated human fH gene therapy in $fH^{m/m}$ mice inhibits alternative pathway complement activation due to the lack of sufficient endogenous mouse fH expression, untreated $fH^{m/m}$ mice have uncontrolled fluid phase alternative pathway complement activation, and as a result they consume plasma C3 and fB (compare Lane 1 of WT with Lanes 2, 4, 6 of three $fH^{m/m}$ mice before gene therapy). One week after $fH^{m/m}$ mice were treated with AAV8-hfH1-4.678.19-20, plasma C3 and fB levels significantly increased compared with pre-treatment levels, suggesting that AAV8-mediated human fH gene therapy inhibited uncontrolled alternative pathway complement activation and C3 and fB consumption. All three mice (F1, F2 and F20) received 3×10^{11} gene copies each via retro-orbital I.V..

FIG 12A provides a schematic domain structure of human factor H variant containing SCR1-4, 6-8, and 17-20, with the locations of N-glycosylatioins sites illustrated by arrows.

FIGs 12B -12C provide the nucleic acid and amino acid sequences of the leader peptide and 11 short consensus repeat (SCR) domains of the fH variant SCR1-4, 6-8 and 17-20 [SEQ ID NO: 45 and 46, respectively].

FIG 13 is the complete cDNA and 5'UTR sequences of the human factor H variant containing the leader peptide and SCR1-4, 6-8 and 17-20 (hfH1-4.678.17-20) (5'UTR is in capital letters) [SEQ ID NO: 47].

FIG 14 is the amino acid sequence of the factor H truncation construct containing the leader peptide (underlined) and SCR1-4, 6-8, and 17-20 (hfH1-4.678.17-20) [SEQ ID NO 48].

FIGs 15A-15C show ELISA detection of hfH1-4.6-8.17-20 protein level in the plasma of 3 fH mutant mice treated with varying doses of AAV8- hfH1-4.678.17-20. The $fH^{m/m}$ mouse is a strain of fH mutant mice that carry premature stop codons at the beginning of SCR19. These mice produce trace amount of truncated fH (lacking SCR19-20) and has uncontrolled fluid phase alternative pathway complement activation and consumption (secondary C3 and fB deficiency). Mice were infected by retro-orbital I.V. with a AAV8 virus containing hfH1-4.678.17-20 at three doses, 1 x 10^{12} gene copies (GC)/mouse, 3 x 10^{11} GC/mouse and 1 x 10^{11} GC/mouse, respectively. Plasma samples were collected for ELISA assay before AAV treatment (Pre) or at one week (W1), two weeks (W2), one month (M1), two months (M2) or 3 months (M3) after AAV treatment. For ELISA assay, the mAb OX-23 was used as a capture antibody (recognizing an epitope in human fH SCR2-3) and biotinylated mAb L20/3 was used as a detection antibody (recognizing human fH SCR19). As shown in the figure, there is no hfH1-4.678.17-20 in the blood of $fH^{m/m}$ mice before AAV-hfH1-4.678.17-20 treatment (Pre), but high level of hfH1-4.678.17-20 was detected after AAV treatment and hfH1-4.678.17-20 expression remained stable for at least 3 months.

FIGs 16A-16C are western blot analysis demonstrating that treatment with AAV8- hfH1-4.678.17-20 gene therapy of $fH^{m/m}$ mice inhibits alternative pathway complement activation. Due to the lack of sufficient endogenous mouse fH expression, untreated $fH^{m/m}$ mice have uncontrolled fluid phase alternative pathway complement activation, and as a result they consume plasma C3 and fB (Lane 1). In three $fH^{m/m}$ mice treated with 1 x10^{12} gene copies (GC)/mouse (FIG 16A), 3 x10^{11} gene copies (GC)/mouse (FIG 16B) and 1 x10^{11} gene copies (GC)/mouse (FIG 16C), respectively, through retro-orbital I.V., alternative pathway complement activation was prevented with corresponding recovery of plasma C3 and fB when the treated mice were examined at one week (W1), one month (M1), 2 months (M2) and 3 months (M3) after AAV8-hfH1-4.678.17-20 gene therapy. In every treatment dosage and time point (Lanes 2, 3, 4, 5), plasma C3 and fB were markedly higher after AAV8- hfH1-4.678.17-20 gene therapy than before treatment (Pre, Lane 1).

FIGs 17A-17C show the nucleic acid and amino acid sequences of the leader peptide and the 20 Short Consensus Repeat (SCR) domains in mice [SEQ ID NO: 79 and

80, respectively]. Amino acid sequences between the defined SCRs are linker sequences that afford fH flexibility.

FIGs 18A-18B provide the nucleic acid and amino acid sequences of the leader peptide and 9 Short Consensus Repeat (SCR) domains of the mouse fH variant [SEQ ID NO: 81 and 82, respectively]. Amino acid sequences between the defined SCRs are linker sequences that afford fH protein flexibility. This variant of mouse fH is used as a surrogate for testing the *in vivo* function of hfH1-4.678.19-20 in subsequent studies.

FIG 19 provides the coding and 5' and 3'-UTR sequences of the mouse factor H truncation construct containing the leader peptide (underlined) and SCR1-4, 6-8, 19-20 (mfH1-4.678.19-20) [SEQ ID NO: 43].

FIG 20 provides the amino acid sequence of the mouse factor H truncation construct containing the leader peptide (underlined) and SCR1-4, 6-8, and 19-20 (mfH1-4.678.19-20) [SEQ ID NO:44].

FIG 21 is a gel showing confirmation of protein expression and stability of mfH1-4.19-20 and mfH1-4.678.19-20. The cDNA sequence of mouse fH truncation variant containing SCR1-4, 678, and 19-20 or that of another fH truncation variant containing SCR1-4, and 19-20 was cloned into a eukaryotic expression vector pCBARBG which was then used to transfect a mouse liver cell line, Hepa1C1C7 cells. Cell culture supernatant was used for western blot analysis to detect truncated mouse fH protein expression. M: molecular weight markers; Lane 1, untransfected Hepa1C1C7 cells (Control); Lane 2 and 3, Hepa1C1C7cells transfected with pCBARBG-mfH1-4.19-20 clone 3 or clone 4; Lanes 5 and 6, Hepa1C1C7cells transfected with pCBARBG-mfH1-4.678.19-20 clone 1 (sense) or clone 2 (antisense).

FIG 22A is a flow chart showing how blood samples were collected and processed for fH protein detection. The $fH^{m/m}$ mouse is a strain of fH mutant mice that carry premature stop codons at the beginning of SCR19. These mice produce trace amount of truncated fH (lacking SCR19-20) and has uncontrolled fluid phase alternative pathway complement activation and consumption (secondary C3 and fB deficiency). Mice were infected by retro-orbital I.V. with an AAV8 virus containing mfH1-4.678.19-20 (3×10^{12} gene copies/mouse) and after one week, blood samples were collected, processed and analyzed as shown in the flow chart.

FIG 22B is a western blot detection of mfH1-4.678.19-20 in the blood of a fH mutant mouse ($fH^{m/m}$) one week after AAV8-mediated fH gene therapy. As shown in the figure, there was no mfH1-4.678.19-20 (approximately 70 kd) in WT and non-treated $fH^{m/m}$ mice. In three virus-infected $fH^{m/m}$ mice, M3, F10, F30 (M indicates male and F indicates female), mfH1-4.678.19-20 was clearly detected.

FIG 23 is a western blot analysis demonstrating that AAV8-mediated fH gene therapy in $fH^{m/m}$ mice prevents uncontrolled alternative pathway complement activation. Due to the lack of sufficient endogenous fH expression, untreated fHm/m mice have uncontrolled fluid phase alternative pathway complement activation, and as a result they consume plasma C3 and fB (compare Lane 1 of WT with Lanes 2, 5, 8 of $fH^{m/m}$ mice before gene therapy). After $fH^{m/m}$ mice were treated with AAV8-mfH1-4.678.19-20, at one week (1W, Lanes 3, 6, 9) and one month (1M, Lanes 4, 7, 10), plasma C3 and fB levels were recovered to WT levels, suggesting that AAV8-mediated fH gene therapy prevented uncontrolled alternative pathway complement activation and C3 and fB consumption, and that the therapeutic effect was evident as early as one week and last at least one month.

FIG 24 is a western blot analysis demonstrating that AAV8-mediated fH gene therapy prevents uncontrolled alternative pathway complement activation in a mouse model of lethal C3 glomerulopathy. In $fH^{m/m}$ mice that are also deficient in properdin ($fH^{m/m}P^{-/-}$), a similar uncontrolled alternative pathway complement activation with C3 and fB consumption occurs. Compared with $fH^{m/m}$ mice, fHm/mP-/- mice develop a lethal form of C3G and they die by the age of 10-12 weeks old. In this experiment, two $fH^{m/m}P^{-/-}$ mice aged around 7-weeks old each were treated with AAV8-mfH1-4.678.19-20 or empty AAV8 vector (pAAV.TBG.rBG) as a control group (Control AAV). One week after AAV8 gene therapy, blood samples were collected and analyzed by western blot for C3 and fB levels. As shown in the panels, compared with blood samples before AAV8 treatment (pre), there was no difference in intact C3 or fB levels one week (1W) after control AAV8 treatment (Lanes 2-5). However, plasma C3 and fB levels in mice one week after treatment with AAV8-mfH1-4.678.19-20 were significantly increased (Lanes 6-9), suggesting uncontrolled alternative pathway complement activation was

inhibited by gene therapy. Mice were treated with AAV8 ($3x10^{12}$ gene copies/mouse) via retro-orbital I.V. injection.

FIG 25 shows long term follow-up of an $fH^{m/m}P^{-/-}$ (M3 from Fig 24)-treated with AAV8-mfH1-4.678.19-20 gene therapy. Western blot analysis of plasma C3 and fB levels before gene therapy (Pre) and at 1 week (1W), 1, 2, 3, 4, 5 and 6 months (1M, 2M, 3M, 4M, 5M, 6M) after treating with AAV8-mfH1-4.678.19-20 showing C3 and fB were persistently elevated to wild-type mouse levels after gene therapy, suggesting that the therapeutic effect was long-lasting.

FIG 26 shows long term follow-up of an $fH^{m/m}P^{-/-}$ (M3 from Fig 24) treated with AAV8-mfH1-4.678.19-20 gene therapy. ELISA analysis of plasma levels of mfH1-4.678.19-20 protein before (Pre) and 1 week, 1, 2, 3, 4, 5 and 6 months (M) after treating with AAV8-mfH1-4.678.19-20 showing that mfH1-4.678.19-20 as a therapeutic protein drug was persistently expressed.

FIG 27 shows the efficacy of AAV8-mfH1-4.678.19-20 gene therapy in preventing renal pathology in C3 glomerulopathy. An $fH^{m/m}P^{-/-}$ mouse treated with control AAV8 vector (mouse M1 from Fig 24) was moribund within 2 weeks of treatment and immunostaining of its kidney showed strong glomerular C3 deposition as previously described for untreated $fH^{m/m}P^{-/-}$ mice (left panels). In contrast, a $fH^{m/m}P^{-/-}$ mouse treated with the AAV8-mfH1-4.678.19-20 vector (M3 from Fig 23) survived and was still healthy at 6 month after treatment, at which time it was sacrificed and analyzed for kidney histology. No glomerular C3 deposition was detected in this mouse (right panels), suggesting C3 glomerulopathy was prevented by AAV8-mfH1-4.678.19-20 gene therapy.

FIGs 28A and 28B demonstrate that AAV8-mfH1-4.678.19-20 gene therapy prevents alternative pathway complement activation caused by membrane regulator dysfunction. In this experiment, mice deficient in two membrane regulators, DAF and Crry, were treated with AAV8-mfH1-4.678.19-20 (retro-orbital route, I.V., $3 x10^{12}$ gene copies/mouse). Plasma samples were collected before and 1 week (1W) after gene therapy to analyze plasma C3 (A) and fB (B) levels by western blot. As shown by the data, the DAF/Crry double mutant mice had excessive alternative pathway complement activation with low C3 and fB levels (Pre). After AAV8-mfH1-4.678.19-20 treatment,

both C3 and fB were restored to wild-type mouse levels, suggesting that AAV8-mfH1-4.678.19-20 treatment can correct pathologies caused by membrane complement regulators. This data suggested that AAV8-mfH1-4.678.19-20 treatment was broadly effective for complement-mediated diseases caused by uncontrolled alternative pathway complement regulation, irrespective of the underlying regulatory mechanism defect. DAF/Crry double mutant mice used in this study is a crossbreed species between DAF knockout mice and a $Crry^{flox/flox}$-Tie-$2Cre^{+}$ mice. Because Tie-2-Cre is expressed in germ cells, it led to germline deletion of Crry gene in some progenies, leading to global Crry deletion.

FIGs 29A - 29B provide a dosage comparison of AAV8-mfH1-4.678.19-20 gene therapy using C3 recovery as a readout. In this experiment, different doses of AAV8-mfH1-4.678.19-20 were administered to $fH^{m/m}$ mice (retro-orbital route, I.V.). Two mice each was given the following dosages: 1×10^{12} gene copies/mouse (M#1, M#2), 3×10^{11} gene copies/mouse (M#3, M#6) and 1×10^{11} gene copies/mouse (M#4, M#5). Western blot was performed to analyze plasma C3 levels before (Pre) and one week (1W) or 1 month (1M) after gene therapy. As shown, all doses tested were able to increase plasma C3 levels when examined at 1W and 1M time points.

FIGs 30A-30B provide a dosage coparison of AAV8-mfH1-4.678.19-20 gene therapy using fB recovery as a readout. The Western analysis was performed essentially as described in FIG 29A-B where C3 was used as a readout. As shown, all doses tested were able to increase plasma fB levels when examined at 1W and 1M time points.

FIG 31 is a schematic diagram showing the gene targeting strategy used to introduce a W to R mutation in SCR20 of mouse fH (position 1206, corresponding to position 1183 in human fH).

FIG 32 shows the survival curves of wild-type littermate mice and mutant mice carrying W1206R mutation in fH. The fH mutant mice developed characteristic pathologies of aHUS and close to half of them died by 30 weeks of age.

FIG 33A shows a comparison of platelet counts in wild-type, heterozygous, and homozygous mutant mice. The homozygous mutant mice showed low platelet counts, suggesting that they were suffering from chronic thrombocytopenia.

FIG 33B shows a comparison of hemoglobin levels in wild-type, heterozygous, and homozygous mutant mice. The homozygous mutant mice show low hemoglobin levels, suggesting that they are suffering from chronic hemolytic anemia.

FIGs 34A-34C show kidney sections of W1206R mutant mice showed pathologies characteristic of aHUS. The pathological features included mesangial expansion and narrowing of capillary lumens (Panel A), thrombi in small vessels as indicated by arrows in Panel A and Panel C. Electron microscopy showed that the glomerular capillary wall exhibited sub-endothelial expansion with fluffy granular electron-lucent material, and formation of double contours and new glomerular basement membrane.

FIGs 35A-35D show that mice carrying W1206R mutation in fH also developed retinal injury and blood clotting in the eye. Compared with normal looking retinas of wild-type mice (FIG 35A), there were many white patches, retinal edema and dilated vessels in the retina of the fH W1206R mutant mouse (FIG 35B). In addition, fluorescien angiography showed the mutant mouse retina was not well perfused as the dye reached all blood vessels in the wild-type mouse eye within 30 seconds (FIG 35C) but it did not reach out to much of the area in the mutant mouse retina even at 4 min (FIG 35D).

FIG 36 shows that mfH1-4.678.19-20 protein was detected by ELISA in the blood of $fH^{W1206R/W1206R}$ mice at 1 month and 2 months after treatment with AAV8-mfH1-4.678.19-20 vector at 3×10^{11}GC/mouse but not in the blood of these mice before AAV gene therapy.

FIGs 37A and 37B are line graphs showing the treatment of $fH^{W1206R/W1206R}$ mice with AAV8-mfH1-4.678.19-20 vector at 3×10^{11} GC/mouse normalized their platelet counts. All 3 $fH^{W1206R/W1206R}$ mice treated with AAV8-mfH1-4.678.19-20 were alive and healthy. Their platelet counts (FIG 37A) and hemoglobin levels (Hb, FIG 37B), increased and were maintained at normal range. In contrast, 1 of 2 $fH^{W1206R/W1206R}$ mice treated with control AAV vector died (at 4 weeks after treatment) and the remaining mouse had consistent low platelet counts and fluctuating hemoglobin level that was below that of mice treated with AAV8-mfH1-4.678.19-20.

DETAILED DESCRIPTION OF THE INVENTION

Novel engineered factor H (fH) genes and protein variants are described herein. These variants are characterized by increased half-life and increased efficacy in treating conditions associated with factor H and other complement disorders.

Delivery of these variants to subjects in need thereof via a number of routes, and particularly by expression *in vivo* mediated by a recombinant vector such as a rAAV vector, are described. Also provided are methods of using these variants in regimens for treating factor H associated disorders. Advantageously, compositions provided herein are useful for simultaneously targeting multiple pathways and/or treating or modulating uncontrolled alternative pathway complement regulation caused by a variety of factors.

As used herein, the term "treating complement factor H disorders" may encompass alleviating, reducing, and/or ameliorating symptoms, and/or preventing the development of additional symptoms associated with complement factor H disorder, which can manifest as several different phenotypes, including asymptomatic, recurrent bacterial infections, and renal failure. This is typically characterized by decreased serum levels of factor H, complement component C3, and a decrease in other terminal complement components, indicating activation of the alternative complement pathway. This disorder is associated with a number of renal diseases with variable clinical presentation and progression, including C3 glomerulopathy and atypical hemolytic uremic syndrome. Also provided herein are compositions and methods for treating one or more of age related macular degeneration (AMD), atypical hemolytic uremic (including, e.g., syndrome microangiopathic haemolytic anemia, thrombocytopenia, acute renal failure), paroxysmal nocturnal hemoglobinuria (PNH), schizophrenia, ischemic stroke, and/or preventing or treating bacterial infections caused by recruitment of bacterial pathogens (e.g., Aspergillus spp.; *Borrelia burgdorferi; B. duttonii; B. recurrentis; Candida albicans; Francisella tularensis; Haemophilus influenzae; Neisseria meningitidis; Streptococcus pyogenes*, or one of the five factor H binding proteins of *B.burgdorferi* (CRASP-1, CRASP-2, CRASP-3, CRASP-4, or CRASP-5), among others.

As used herein, the term "treating complement associated disorders" includes alleviating, reducing, and/or ameliorating symptoms, both of the complement factor H disorders identified above, but also other disorders associated with uncontrolled

alternative pathway complement regulation. More particularly, the data provided herein suggests that at least one AAV-mediated fH variant is broadly effective for complement-mediated diseases caused by uncontrolled alternative pathway complement regulation, irrespective of the underlying regulatory mechanism defect. *See, e.g,* FIG 23.

"Complement-mediated disorders" may encompass symptoms associated with complement dysregulation which can manifest as several different phenotypes, including asymptomatic, recurrent bacterial infections, and various tissue injuries including but not limited to renal diseases. Unless otherwise specified, both homozygous subjects and heterozygous subjects are encompassed within this definition. Complement dysregulation is typically caused by loss of function mutations in, or auto-antibodies against, complement regulatory proteins including but not limited to fH, factor I (fI) and membrane cofactor protein (MCP) or by gain of function mutations in other complement proteins including but not limited to C3 and factor B (fB). Complement dysregulation is typically, though not always, characterized by decreased serum levels of factor H, complement component C3, fB and a decrease in other terminal complement components, indicating activation of the alternative and/or the terminal complement pathway. Complement-mediated pathologies that can be treated by the present invention of composition and method include but are not limited to the following diseases with variable clinical presentation and progression: C3 glomerulopathy (formally called membranoproliferative glomerulonephritis type II or MPGNII), of which there are two known forms - dense deposit disease (DDD) and C3 glomerulonephritis (C3GN); thrombotic microangiopathy (TMA) including but not limited to atypical hemolytic uremic syndrome (aHUS), Shiga-like toxin-producing *E. coli* HUS (STEC-HUS) and thrombotic thrombocytopenia purpura (TTP); retinal degenerative eye disease including age related macular degeneration (AMD), RPE degeneration, chorioretinal degeneration, photoreceptor degeneration, paroxysmal nocturnal hemoglobinuria (PNH), ischemia reperfusion injury of all organs and settings, rheumatoid arthritis, hemodialysis, diabetic nephropathy, diabetic vasculopathy, asthma, systemic lupus erythematosus (SLE), ischemic stroke, abdominal aortic aneurysm (AAA), anti-neutrophil cytoplasmic antibody (ANCA) mediated vasculitis (ANCA vasculitis), ANCA-mediated hemorrhagic lung injury and disease, ANCA glomerulonephritis, graft versus host disease (GvHD),

acute or delay graft rejection in organ transplantation, Crohn's disease, psoriasis, multiple sclerosis, anti-phospholipid syndrome, preeclampsia, atherosclerosis, neuromyelitis optica (NMO), autoimmune skin-blistering disease, Bullous pemphigoid (BP), Alzheimer's disease (AD), as well as bacterial infections caused by recruitment of bacterial pathogens (e.g., *Aspergillus spp.*; *Borrelia burgdorferi*; *B. duttonii*; *B. recurrentis*; *Candida albicans*; *Francisella tularensis; Haemophilus influenzae; Neisseria meningitidis; Streptococcus pyogenes.* Other examples of such disorders are discussed in more details below.

The amino acid sequence of the mature "wild-type" human complement factor H (isoform 1) is provided herein as http://www.uniprot.org/uniprot/ P08603 and serves as a reference for the amino acid numbering of the hfH isoform 1 [reproduced in SEQ ID NO: 39]. The leader sequence is located at amino acids 1 to 18 of factor H, with reference to SEQ ID NO: 39. The amino acid sequence of the leader is provided in SEQ ID NO: 2. The mature (secreted) hfH protein is located at amino acids 19 to 1231, with reference to SEQ ID NO: 39. There are alternative methods of determining the location of the 20 short complement repeats (SCRs). The location of the domains used in the experiments provided below is annotated in FIG 1 and is based on the numbering used in C. Estaller et al, Eur J Immunol. 1991 Mar; 21(3):799-802.

The amino acid sequence of the wild-type human complement factor H, isoform 1, is reproduced in SEQ ID NO: 39. The features section of SEQ ID NO: 1 also illustrates an alternative system for identifying the start/stop of each of the 20 Short Consensus Repeat (SCR) domains. In this system, linker sequences are not present between each of the SCRs.

Optionally, an engineered hfH variant provided herein may have a heterologous leader sequence substituted for the native hfH leader sequence. Additionally, or optionally, another hfH isoform (*e.g.,* isoform 2), the sequence of which are available, *e.g.*, from http://www.uniprot.org/uniprot/ P08603, and/or one of the natural amino acid variants therein which are not associated with a disorder. See, SEQ ID NO: 40. In the following descriptions, substitutions may be written as (first amino acid identified by single letter code)- residue position # - (second amino acid identified by single letter code) whereby the first amino acid is the substituted amino acid and the second amino

acid is the substituting amino acid at the specified position with reference to isoform 1; however, by conventional alignment steps, the corresponding amino acid residues identified herein with respect to the numbering of isoform 1 can be located in isoform 2 and non-disease causing natural variants of the SCRs of isoform 1 or 2 of fH.

As used herein, when reference is made to SCR #-##, the domains are inclusive of the endpoints and is the same as "SCR#, . . . SCR##". In certain embodiments, periods are used between the domains. For example, SCR1-4, refers to "SCR1, SCR2, SCR3, and SCR4" and is the same as "SCR1,2,3,4" or "SCR1.2.3.4.". SCR19-20, refers to SCR19 and SCR20 and is the same as "SCR19,20". For example, "SCR6-8", "SCR6.7.8" and "SCR6,7,8" refer to the same domains.

As used herein, the term "functional fH variant" includes fH variants which are characterized by having complement regulating activity (cofactor activity) located in SCR1-4 and optionally, a functional C3b-binding and GAG-binding ability (located within wild-type SCR7 and SCR19-20) characteristic of wild-type fH. In some embodiments, the engineered fH variants have more than 100% of wild-type fH cofactor activity and/or GAG-binding ability. *See, e.g.*, Figure 8 and the examples below which demonstrate that a fH variant described herein has GAG- and C3b-binding activity statistically higher than full-length human fH, *e.g.*, about 10% to 40% higher. In another embodiment, the engineered fH variant has less than about 95% to about 100% of wild-type functional fH. For example, an engineered fH variant may have at least 50% of the cofactor activity present in functional wild-type fH, and more desirably at least about 60%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95% , or at least about 99%. In another embodiment, the engineered fH variant may alternatively or additionally have at least at least 50% of the GAG-binding ability of functional fH, and more desirably at least about 60%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95% , or at least about 99%. Methods of determining cofactor activity, binding and/or determining increased circulating half-life as compared to the hfH proteins are known in the art, and at least one these assays is illustrated in the examples below.

Examples of functional fH variants include those having SCR1-4 and 19-20 of the fH protein, with one or more of an SCR7, SCR17 or SCR18 domain. Further variants

include those having one or more of SCR6, SCR8, SCR16, SCR17, SCR18, or fragments thereof, and combinations thereof. For example, such variants may include, *e.g.,* fH SCR1-4, 6-8,19-20; fH SCR1-4, 6-8,18-20; fH SCR1-4, 6-8,17-20; fH SCR1-4, 6-7,19-20; fH SCR1-4,6-7,18-20; fH SCR1-4,6-7,17-20; fH SCR1-4, 7- 8,19-20; fH SCR1-4,7-8,18-20; fH SCR1-4,7-8,17-20; fH SCR1-4, 7,19-20; fH SCR1-4,7,18-20; fH SCR1-4,7,17-20; SCR1-4, 17, 19-20; SCR1-4, 18-20; SCR1-4, 17-20 and/or fH SCR1-4,7,16-20, among others. In certain embodiments, the hfH variant further comprises additional hfH SCRs, *e.g.*, SCR 6, SCR8, SCR16, or combinations thereof. In preferred embodiments, hfH SCR5 is absent. However, in certain embodiments, hfH SCR5 may be present in whole or a fraction thereof. In certain embodiments, hfH SCR9, SCR10, SCR11, SCR12, SCR13, SCR14, and/or SCR15 are absent, or are at least functionally deleted. Optionally, one or more of the SCRs in these variants may be a "functional fragment" of the SCRs, rather than a full-length SCR as shown in FIG 1 or the features of SEQ ID NO: 1. By "functional fragment" is meant an amino acid sequence (or coding sequence therefor) less than the full-length SCR which is characterized by having one or more of complement inhibiting activity, the ability to bind, heparin, and/or C3b-binding activity.

These and other variants may include other fH sequences. For example, when expressed from a viral vector the coding sequence of the fH variant also includes a leader sequence. Such a leader sequence may be an fH leader. Optionally, the leader sequence can be from another source, *e.g.*, an IL-2 leader, [see, *e.g.*, the index of mammalian leader sequences identified in http://www.signalpeptide.de/], incorporated by reference herein. In one embodiment, the leader sequence selected is less than about 26 amino acids in length (*e.g.*, from about 1 to about 26 amino acids), more preferably less than 20 amino acids (from about 1 to about 20 amino acids), and most preferably, less than about 18 amino acids in length (from about 1 to about 18 amino acids). By "functional deletion" is meant an amino acid sequence (or coding sequence therefor) which lacks complement inhibiting activity, the C3b-binding activity, and optionally also further lacks heparin binding activity.

With the variants, domains may be located immediately adjacent to one another (*e.g.*, the carboxy terminus of one domain may immediately follow the amino terminus

of the preceding domain). Alternatively, one or more of the SCR domains may have a linker composed of one to about 12 to 18 amino acids located between them. For example, a variant may contain SCR1-(L1)- SCR2-(L2)-SCR3-(L3)-SCR4-(L4)-(SCR6-(L4'))-SCR7-(L5)-(SCR8-(L5'))-(SCR16-(L5"))-(SCR17-(L5"'))-(SCR18-(L5""))-SCR19-(L6)-SCR20, wherein the () indicate optional component, "L" refers to a linker, and each of L1, L2, L3, L4, L4', L5, L5', L5", L5"', L5"", and L6 may be absent or independently selected from an amino acid sequence of about 1 to about 12-18 amino acids. In other words, where a variant contains multiple linkers, each of the linkers may have the same sequence or a different sequence. In certain embodiments, a variant contains at least one, at least two, at least three, at least four, at least five linkers, at least six linkers. Examples of suitable linkers include the natural linkers identified in FIG1 or FIG17, SEQ ID NO: 4, 6, 8, 10, 12, 15, 18, 20, 22, 24, 26, 28, 30, 32, 34 and 36, or synthetic linkers. Each of these wild-type linkers may be located in their native position. Alternatively, one or more of these wild-type linkers may be used in a different linker position, or in multiple different linker positions.

Optionally, one or more of these linkers may be fH sequences and are independently selected. Alternatively, one or more of the linkers may be heterologous to fH, *e.g.*, from a different source, whether artificial, synthetic, or from a different protein which confers suitable flexibility to the fH variant. Examples of other suitable linkers may include, *e.g.*, a poly Gly linker and other linkers providing suitable flexibility (e.g., http://parts.igem.org/Protein_domains/Linker), which is incorporated by reference herein. In certain embodiments, the linkers lack any fH function.

The term "amino acid substitution" and its synonyms described above are intended to encompass modification of an amino acid sequence by replacement of an amino acid with another, substituting, amino acid. The substitution may be a conservative substitution. It may also be a non-conservative substitution. The term conservative, in referring to two amino acids, is intended to mean that the amino acids share a common property recognized by one of skill in the art. For example, amino acids having hydrophobic nonacidic side chains, amino acids having hydrophobic acidic side chains, amino acids having hydrophilic nonacidic side chains, amino acids having hydrophilic acidic side chains, and amino acids having hydrophilic basic side chains.

Common properties may also be amino acids having hydrophobic side chains, amino acids having aliphatic hydrophobic side chains, amino acids having aromatic hydrophobic side chains, amino acids with polar neutral side chains, amino acids with electrically charged side chains, amino acids with electrically charged acidic side chains, and amino acids with electrically charged basic side chains. Both naturally occurring and non-naturally occurring amino acids are known in the art and may be used as substituting amino acids in embodiments. Methods for replacing an amino acid are well known to the skilled in the art and include, but are not limited to, mutations of the nucleotide sequence encoding the amino acid sequence. Reference to "one or more" herein is intended to encompass the individual embodiments of, for example, 1, 2, 3, 4, 5, 6, or more.

In addition to the fH protein variants provided herein, nucleic acid sequences encoding these fH protein variants are provided. The coding sequences for these variants may be from wild-type sequences of the leader sequence and/or one or more SCRs of isoform 1, isoform 2, or non-disease associated variants. Alternatively or additionally, web-based or commercially available computer programs, as well as service based companies may be used to back translate the amino acids sequences of the leader sequence, and/or one or more of the SCRs to nucleic acid coding sequences, including both RNA and/or cDNA. *See, e.g.,* backtranseq by EMBOSS, http://www.ebi.ac.uk/Tools/st/; Gene Infinity (http://www.geneinfinity.org/sms-/sms_backtranslation.html); ExPasy (http://www.expasy.org/tools/). In one embodiment, the RNA and/or cDNA coding sequences are designed for optimal expression in human cells.

Codon-optimized coding regions can be designed by various different methods. This optimization may be performed using methods which are available on-line, published methods, or a company which provides codon optimizing services. One codon optimizing method is described, *e.g.,* in WO 2015/012924 A2, which is incorporated by reference herein. Briefly, the nucleic acid sequence encoding the product is modified with synonymous codon sequences. Suitably, the entire length of the open reading frame (ORF) for the product is modified. However, in some embodiments, only a fragment of the ORF may be altered. By using one of these methods, one can apply the frequencies to

any given polypeptide sequence, and produce a nucleic acid fragment of a codon-optimized coding region which encodes the polypeptide.

The terms "percent (%) identity", "sequence identity", "percent sequence identity", or "percent identical" in the context of nucleic acid sequences refers to the bases in the two sequences which are the same when aligned for correspondence. The length of sequence identity comparison may be over the full-length of the genome, the full-length of a gene coding sequence, or a fragment of at least about 500 to 5000 nucleotides, or as desired. However, identity among smaller fragments, *e.g.* of at least about nine nucleotides, usually at least about 20 to 24 nucleotides, at least about 28 to 32 nucleotides, at least about 36 or more nucleotides, may also be desired. Multiple sequence alignment programs are also available for nucleic acid sequences. Examples of such programs include, “Clustal W”, “CAP Sequence Assembly”, “BLAST”, “MAP”, and “MEME”, which are accessible through Web Servers on the internet. Other sources for such programs are known to those of skill in the art. Alternatively, Vector NTI utilities are also used. There are also a number of algorithms known in the art that can be used to measure nucleotide sequence identity, including those contained in the programs described above. As another example, polynucleotide sequences can be compared using Fasta™, a program in GCG Version 6.1. Fasta™ provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. For instance, percent sequence identity between nucleic acid sequences can be determined using Fasta™ with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) as provided in GCG Version 6.1, herein incorporated by reference.

The terms "percent (%) identity", "sequence identity", "percent sequence identity", or "percent identical" in the context of amino acid sequences refers to the residues in the two sequences which are the same when aligned for correspondence. Percent identity may be readily determined for amino acid sequences over the full-length of a protein, polypeptide, about 32 amino acids, about 330 amino acids, or a peptide fragment thereof or the corresponding nucleic acid sequence coding sequencers. A suitable amino acid fragment may be at least about 8 amino acids in length, and may be up to about 700 amino acids. Generally, when referring to "identity", "homology", or

"similarity" between two different sequences, "identity", "homology" or "similarity" is determined in reference to "aligned" sequences. "Aligned" sequences or "alignments" refer to multiple nucleic acid sequences or protein (amino acids) sequences, often containing corrections for missing or additional bases or amino acids as compared to a reference sequence. Alignments are performed using any of a variety of publicly or commercially available Multiple Sequence Alignment Programs. Sequence alignment programs are available for amino acid sequences, *e.g.,* the "Clustal X", "MAP", "PIMA", "MSA", "BLOCKMAKER", "MEME", and "Match-Box" programs. Generally, any of these programs are used at default settings, although one of skill in the art can alter these settings as needed. Alternatively, one of skill in the art can utilize another algorithm or computer program which provides at least the level of identity or alignment as that provided by the referenced algorithms and programs. See, *e.g.,* J. D. Thomson et al, Nucl. Acids. Res., "A comprehensive comparison of multiple sequence alignments", 27(13):2682-2690 (1999).

In one embodiment, the nucleic acid sequences encoding the fH variants (*e.g.,* hfH variant gene) described herein are engineered into any suitable genetic element, *e.g.,* naked DNA, phage, transposon, cosmid, RNA molecule (*e.g.,* mRNA), episome, *etc.,* which transfers the hfH sequences carried thereon to a host cell, *e.g.,* for generating nanoparticles carrying DNA or RNA, viral vectors in a packaging host cell and/or for delivery to a host cells in subject. In one embodiment, the genetic element is a plasmid. The selected genetic element may be delivered by any suitable method, including transfection, electroporation, liposome delivery, membrane fusion techniques, high velocity DNA-coated pellets, viral infection and protoplast fusion. The methods used to make such constructs are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques. See, *e.g.,* Green and Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY (2012).

As used herein, an "expression cassette" refers to a nucleic acid molecule which comprises the hfH variant coding sequences, promoter, and may include other regulatory sequences therefor (e.g., 5' and/or 3' UTR sequences), which cassette may be engineered into a genetic element and/or packaged into the capsid of a viral vector (*e.g.,* a viral

particle). Typically, such an expression cassette for generating a viral vector contains the hfH sequences described herein flanked by packaging signals of the viral genome and other expression control sequences such as those described herein.

The expression cassette typically contains a promoter sequence as part of the expression control sequences. The illustrative plasmid and vector described herein uses the chicken beta-actin. Alternatively, another constitutive promoter may be selected. In certain embodiments, de-targeting of undesirable target cells may be achieved by use of appropriate vector elements, *e.g.*, microRNAs. Additionally or alternatively, the vector selected may have preferential targeting for the desired tissue, *e.g.*, an AAV8, AAV9, or AAVrh10 for liver, an AAV8, AAV1, or other AAV for eye, or the like.

However, targeting the vector to a desired tissue may be desirable for maximizing expression of the protein. And as such, a liver-specific promoter may be selected. Examples of suitable promoters include, thyroxin binding globulin (TBG), alpha 1 anti-trypsin (A1AT); human albumin Miyatake et al., J. Virol., 71:5124 32 (1997), humAlb; and hepatitis B virus core promoter, Sandig *et al.*, Gene Ther., 3:1002 9 (1996)]. TTR minimal enhancer/promoter, alpha-antitrypsin promoter, LSP (845 nt) 25 (requires intron-less scAAV). Alternatively, other liver-specific promoters may be used [see, *e.g.*, The Liver Specific Gene Promoter Database, Cold Spring Harbor, http://rulai.schl.edu/LSPD. Alternatively, where targeting to another tissue is desired, a different tissue-specific promoter may be selected. The promoter may be derived from any species. For example, for use in the eye, *e.g.*, a retinal pigmented epithelium (RPE) promoter or a photoreceptor promoter may be selected. In another embodiment, the promoter is the human G-protein-coupled receptor protein kinase 1 (GRK1) promoter (Genbank Accession number AY327580). In another embodiment, the promoter is a 292 nt fragment (positions 1793-2087) of the GRK1 promoter (See also, Beltran et al, Gene Therapy 2010 17:1162-74, which is hereby incorporated by reference herein). In another preferred embodiment, the promoter is the human interphotoreceptor retinoid-binding protein proximal (IRBP) promoter. In another embodiment, promoter is the native promoter for the gene to be expressed. In one embodiment, the promoter is the RPGR proximal promoter (Shu et al, IOVS, May 2012, which is incorporated by reference herein). Other promoters useful in the invention include, without limitation, the rod opsin

promoter, the red-green opsin promoter, the blue opsin promoter, the cGMP-β-phosphodiesterase promoter, the mouse opsin promoter (Beltran et al 2010 cited above), the rhodopsin promoter (Mussolino et al, Gene Ther, July 2011, 18(7):637-45); the alpha-subunit of cone transducin (Morrissey et al, BMC Dev, Biol, Jan 2011, 11:3); beta phosphodiesterase (PDE) promoter; the retinitis pigmentosa (RP1) promoter (Nicord et al, J. Gene Med, Dec 2007, 9(12):1015-23); the NXNL2/NXNL1 promoter (Lambard et al, PLoS One, Oct. 2010, 5(10):e13025), the RPE65 promoter; the retinal degeneration slow/peripherin 2 (*Rds/perph2*) promoter (Cai et al, Exp Eye Res. 2010 Aug;91(2):186-94); and the VMD2 promoter (Kachi et al, Human Gene Therapy, 2009 (20:31-9)). Examples of photoreceptor specific promoters include, without limitation, the rod opsin promoter, the red-green opsin promoter, the blue opsin promoter, the inter photoreceptor binding protein (IRBP) promoter and the cGMP-β-phosphodiesterase promoter. Alternatively, other promoters, such as viral promoters, constitutive promoters, regulatable promoters [*see, e.g.,* WO 2011/126808 and WO 2013/04943], or a promoter responsive to physiologic cues may be used may be utilized in the vectors described herein.

In addition to a promoter, an expression cassette and/or a vector may contain other appropriate transcription initiation, termination, enhancer sequences, efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (*e.g.,* Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. Examples of suitable polyA sequences include, *e.g.*, SV40, bovine growth hormone (bGH), rabbit beta globulin, and TK polyA. Examples of suitable enhancers include, *e.g.*, the alpha fetoprotein enhancer, the TTR minimal promoter/enhancer, LSP (TH-binding globulin promoter/alpha1-microglobulin/bikunin enhancer), amongst others.

These control sequences are "operably linked" to the fH gene sequences. As used herein, the term "operably linked" refers to both expression control sequences that are contiguous with the gene of interest and expression control sequences that act *in trans* or at a distance to control the gene of interest.

The expression cassette may be engineered onto a plasmid which is used for drug delivery or for production of a viral vector. Suitable viral vectors are preferably replication-defective and selected from amongst those which target ocular cells. Viral vectors may include any virus suitable for gene therapy may be used, including but not limited to adenovirus; herpes virus; lentivirus; retrovirus; parvovirus, *etc.*

Suitably, where one of these vectors is generated, it is produced as a replication-defective viral vector. A "replication-defective virus" or "viral vector" refers to a synthetic or recombinant viral particle in which an expression cassette containing a gene of interest is packaged in a viral capsid or envelope, where any viral genomic sequences also packaged within the viral capsid or envelope are replication-deficient; *i.e.*, they cannot generate progeny virions but retain the ability to infect target cells. In one embodiment, the genome of the viral vector does not include genes encoding the enzymes required to replicate (the genome can be engineered to be "gutless" - containing only the transgene of interest flanked by the signals required for amplification and packaging of the artificial genome), but these genes may be supplied during production. Therefore, it is deemed safe for use in gene therapy since replication and infection by progeny virions cannot occur except in the presence of the viral enzyme required for replication.

In one embodiment, the viral vector is an adeno-associated virus (AAV). An adeno-associated virus (AAV) viral vector is an AAV DNase-resistant particle having an AAV protein capsid into which is packaged nucleic acid sequences for delivery to target cells. An AAV capsid is composed of 60 capsid protein subunits, VP1, VP2, and VP3, that are arranged in an icosahedral symmetry in a ratio of approximately 1:1:10 to 1:1:20, depending upon the selected AAV.

The studies described herein utilize AAV8 as an illustrative vector. As used herein, "AAV8 capsid" refers to the AAV8 capsid having the encoded amino acid sequence of GenBank accession:YP_077180, which is incorporated by reference herein. Some variation from this encoded sequence is encompassed by the present invention, which may include sequences having about 99% identity to the referenced amino acid sequence in GenBank accession:YP_077180; US Patent 7,282,199, 7,790,449; 8,319,480; 8,962,330; US 8,962,332, (*i.e.*, less than about 1% variation

from the referenced sequence). In another embodiment, the AAV8 capsid may have the VP1 sequence of the AAV8 variant described in WO2014/124282, which is incorporated by reference herein. Methods of generating the capsid, coding sequences therefore, and methods for production of rAAV viral vectors have been described. See, *e.g.*, Gao, et al, Proc. Natl. Acad. Sci. U.S.A. 100 (10), 6081-6086 (2003), US 2013/0045186A1, and WO 2014/124282. In certain embodiments, an AAV8 variant which shows tropism for the desired target cell, *e.g.*, liver, photoreceptors, RPE or other ocular cells is selected. For example, an AAV8 capsid may have Y447F, Y733F and T494V mutations (also called "AAV8(C&G+T494V)" and "rep2-cap8(Y447F+733F+T494V)"), as described by Kay et al, Targeting Photoreceptors via Intravitreal Delivery Using Novel, Capsid-Mutated AAV Vectors, PLoS One. 2013; 8(4): e62097. Published online 2013 Apr 26, which is incorporated herein by reference. See, *e.g.*, Mowat et al, Tyrosine capsid-mutant AAV vectors for gene delivery to the canine retina from a subretinal or intravitreal approach, Gene Therapy 21, 96-105 (January 2014), which is incorporated herein by reference. In another embodiment, the AAV capsid is an AAV8bp capsid, which preferentially targets bipolar cells. See, WO 2014/024282, which is incorporated herein by reference.

Other AAV serotypes may be selected as sources for capsids of AAV viral vectors (DNase resistant viral particles) including, *e.g.*, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV6.2, AAV7, AAV8, AAV9, rh10, AAVrh64R1, AAVrh64R2, rh8 [See, *e.g.*, US Published Patent Application No. 2007-0036760-A1; US Published Patent Application No. 2009-0197338-A1; EP 1310571]. See also, WO 2003/042397 (AAV7 and other simian AAV), US Patent 7790449 and US Patent 7282199 (AAV8), WO 2005/033321 and US 7,906,111 (AAV9), and WO 2006/110689], and rh10 [WO 2003/042397], variants thereof, or yet to be discovered, or a recombinant AAV based thereon, may be used as a source for the AAV capsid. These documents also describe other AAV which may be selected for generating AAV and are incorporated by reference. In some embodiments, an AAV cap for use in the viral vector can be generated by mutagenesis (i.e., by insertions, deletions, or substitutions) of one of the aforementioned AAV Caps or its encoding nucleic acid. In some embodiments, the AAV capsid is chimeric, comprising domains from two or three or four or more of the

aforementioned AAV capsid proteins. In some embodiments, the AAV capsid is a mosaic of VPl, VP2, and VP3 monomers from two or three different AAVs or recombinant AAVs. In some embodiments, a rAAV composition comprises more than one of the aforementioned Caps.

For packaging an expression cassette into virions, the ITRs are the only AAV components required in *cis* in the same construct as the gene. In one embodiment, the coding sequences for the replication (rep) and/or capsid (cap) are removed from the AAV genome and supplied in *trans* or by a packaging cell line in order to generate the AAV vector. For example, as described above, a pseudotyped AAV may contain ITRs from a source which differs from the source of the AAV capsid. Additionally or alternatively, a chimeric AAV capsid may be utilized. Still other AAV components may be selected. Sources of such AAV sequences are described herein and may also be isolated or obtained from academic, commercial, or public sources (*e.g.*, the American Type Culture Collection, Manassas, VA). Alternatively, the AAV sequences may be obtained through synthetic or other suitable means by reference to published sequences such as are available in the literature or in databases such as, *e.g.*, GenBank®, PubMed®, or the like.

The minimal sequences required to package an expression cassette into an AAV viral particle are the AAV 5' and 3' ITRs, which may be of the same AAV origin as the capsid, or which are of a different AAV origin (to produce an AAV pseudotype). In one embodiment, the ITR sequences from AAV2, or the deleted version thereof (ΔITR), are used for convenience and to accelerate regulatory approval. However, ITRs from other AAV sources may be selected. Where the source of the ITRs is from AAV2 and the AAV capsid is from another AAV source, the resulting vector may be termed pseudotyped. Typically, an expression cassette for an AAV vector comprises an AAV 5' ITR, the coding sequences and any regulatory sequences, and an AAV 3' ITR. However, other configurations of these elements may be suitable. A shortened version of the 5' ITR, termed ΔITR, has been described in which the D-sequence and terminal resolution site (trs) are deleted. In other embodiments, the full-length AAV 5' and 3' ITRs are used.

The abbreviation "sc" refers to self-complementary. "Self-complementary AAV" refers a plasmid or vector having an expression cassette in which a coding region carried

by a recombinant AAV nucleic acid sequence has been designed to form an intra-molecular double-stranded DNA template. Upon infection, rather than waiting for cell mediated synthesis of the second strand, the two complementary halves of scAAV will associate to form one double stranded DNA (dsDNA) unit that is ready for immediate replication and transcription. See, *e.g.*, D M McCarty et al, "Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis", Gene Therapy, (August 2001), Vol 8, Number 16, Pages 1248-1254. Self-complementary AAVs are described in, *e.g.*, U.S. Patent Nos. 6,596,535; 7,125,717; and 7,456,683, each of which is incorporated herein by reference in its entirety.

Methods for generating and isolating AAV viral vectors suitable for delivery to a subject are known in the art. See, *e.g.*, US Patent 7790449; US Patent 7282199; WO 2003/042397; WO 2005/033321, WO 2006/110689; and US 7588772 B2]. In a one system, a producer cell line is transiently transfected with a construct that encodes the transgene flanked by ITRs and a construct(s) that encodes rep and cap. In a second system, a packaging cell line that stably supplies rep and cap is transiently transfected with a construct encoding the transgene flanked by ITRs. In each of these systems, AAV virions are produced in response to infection with helper adenovirus or herpesvirus, requiring the separation of the rAAVs from contaminating virus. More recently, systems have been developed that do not require infection with helper virus to recover the AAV - the required helper functions (*i.e.*, adenovirus E1, E2a, VA, and E4 or herpesvirus UL5, UL8, UL52, and UL29, and herpesvirus polymerase) are also supplied, *in trans*, by the system. In these newer systems, the helper functions can be supplied by transient transfection of the cells with constructs that encode the required helper functions, or the cells can be engineered to stably contain genes encoding the helper functions, the expression of which can be controlled at the transcriptional or posttranscriptional level. In yet another system, the transgene flanked by ITRs and rep/cap genes are introduced into insect cells by infection with baculovirus-based vectors. For reviews on these production systems, see generally, *e.g.*, Zhang et al., 2009, "Adenovirus-adeno-associated virus hybrid for large-scale recombinant adeno-associated virus production," Human Gene Therapy 20:922-929, the contents of each of which is incorporated herein

by reference in its entirety. Methods of making and using these and other AAV production systems are also described in the following U.S. patents, the contents of each of which is incorporated herein by reference in its entirety: 5,139,941; 5,741,683; 6,057,152; 6,204,059; 6,268,213; 6,491,907; 6,660,514; 6,951,753; 7,094,604; 7,172,893; 7,201,898; 7,229,823; and 7,439,065. See generally, *e.g.*, Grieger & Samulski, 2005, "Adeno-associated virus as a gene therapy vector: Vector development, production and clinical applications," Adv. Biochem. Engin/Biotechnol. 99: 119-145; Buning et al., 2008, "Recent developments in adeno-associated virus vector technology," J. Gene Med. 10:717-733; and the references cited below, each of which is incorporated herein by reference in its entirety. The methods used to construct any embodiment of this invention are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques. See, *e.g.*, Green and Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY (2012). Similarly, methods of generating rAAV virions are well known and the selection of a suitable method is not a limitation on the present invention. See, *e.g.*, K. Fisher et al, (1993) J. Virol., 70:520-532 and US Patent No. 5,478,745.

Optionally, the fH genes described herein may be delivered via viral vectors other than rAAV. Such other viral vectors may include any virus suitable for gene therapy may be used, including but not limited to adenovirus; herpes virus; lentivirus; retrovir*us; etc.* Suitably, where one of these other vectors is generated, it is produced as a replication-defective viral vector.

A "replication-defective virus" or "viral vector" refers to a synthetic or artificial viral particle in which an expression cassette containing a gene of interest is packaged in a viral capsid or envelope, where any viral genomic sequences also packaged within the viral capsid or envelope are replication-deficient; *i.e.*, they cannot generate progeny virions but retain the ability to infect target cells. In one embodiment, the genome of the viral vector does not include genes encoding the enzymes required to replicate (the genome can be engineered to be "gutless" - containing only the transgene of interest flanked by the signals required for amplification and packaging of the artificial genome), but these genes may be supplied during production. Therefore, it is deemed safe for use

in gene therapy since replication and infection by progeny virions cannot occur except in the presence of the viral enzyme required for replication.

The pharmaceutical compositions described herein are designed for delivery to subjects in need thereof by any suitable route or a combination of different routes, *e.g.*, direct delivery to the liver (optionally via intravenous, via the hepatic artery, or by transplant), oral, inhalation, intranasal, intratracheal, intraarterial, intraocular, intravenous, intramuscular, subcutaneous, intradermal, and other parental routes of administration. The viral vectors described herein may be delivered in a single composition or multiple compositions. Optionally, two or more different AAV may be delivered, or multiple viruses [see, *e.g.*, WO 2011/126808 and WO 2013/049493]. In another embodiment, multiple viruses may contain different replication-defective viruses (*e.g.,* AAV and adenovirus).

The replication-defective viruses can be formulated with a physiologically acceptable carrier for use in gene transfer and gene therapy applications. In the case of AAV viral vectors, quantification of the genome copies (GC) may be used as the measure of the dose contained in the formulation. Any method known in the art can be used to determine the genome copy (GC) number of the replication-defective virus compositions of the invention. One method for performing AAV GC number titration is as follows: purified AAV vector samples are first treated with DNase to eliminate un-encapsidated AAV genome DNA or contaminating plasmid DNA from the production process. The DNase resistant particles are then subjected to heat treatment to release the genome from the capsid. The released genomes are then quantitated by real-time PCR using primer/probe sets targeting specific region of the viral genome (usually poly A signal).

Also, the replication-defective virus compositions can be formulated in dosage units to contain an amount of replication-defective virus that is in the range of about 1.0 x 10^9 GC to about 1.0 x 10^{15} GC (to treat an average subject of 70 kg in body weight), and preferably 1.0 x 10^{12} GC to 1.0 x 10^{14} GC for a human patient. In another embodiment, the dose is less than about 1.5 x 10^{11} GC/kg. For example, the dose of AAV virus may be about 1 x 10^9 GC, about 5 X 10^9 GC, about 1 X 10^{10} GC, about 5 X

10^{10} GC, or about 1 X 10^{11} GC. In another example, the variants may be delivered in an amount of about 0.001 mg to about 10 mg/kg.

The above-described recombinant vectors may be delivered to host cells according to published methods. The rAAV, preferably suspended in a physiologically compatible carrier, may be administered to a human or non-human mammalian subject. Suitable carriers may be readily selected by one of skill in the art in view of the indication for which the transfer virus is directed. For example, one suitable carrier includes saline, which may be formulated with a variety of buffering solutions (*e.g.*, phosphate buffered saline). Other exemplary carriers include sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, peanut oil, sesame oil, and water. The selection of the carrier is not a limitation of the present invention.

Optionally, the compositions of the invention may contain, in addition to the rAAV and/or variants and carrier(s), excipients, including other non-active conventional pharmaceutical ingredients, such as preservatives, chemical stabilizers, suspending agents, and/or surfactants. Suitable exemplary preservatives include chlorobutanol, potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, and parachlorophenol. Suitable chemical stabilizers include gelatin and albumin. Optionally, for protein-based or antibody-based compositions, excipients suitable for solid compositions may be selected, including, *e.g.*, fillers, beads, bulking agents, disintegrants, glidants, flavorants, colorants, or other components.

The viral vectors and other constructs described herein may be used in preparing a medicament for delivering a fH variant to a subject in need thereof, supplying fH variant having an increased half-life to a subject, and/or for treating complement related disorders.

A course of treatment may optionally involve repeat administration of the same viral vector (*e.g.*, an AAV8 vector) or a different viral vector (*e.g.*, an AAV8 and an AAVrh10). For example, where targeted to the liver, repeat administration may be desirable over 18 months, 2 years, or a longer time period due to dilution of expression caused by natural hepatocyte proliferation. Still other combinations of viral and protein-based treatment may be selected using the viral vectors described herein. Optionally, the composition described herein may be combined in a regimen involving other anti-

complement drugs (*e.g.,* monoclonal antibodies, *etc*), or protein-based therapies (including, *e.g.,* delivery of a composition containing one or more fH variants as described herein).

For example, an engineered hfH variant as described herein may be delivered in protein form. Optionally, when delivered to a subject in protein form, a fH variant may have a leader sequence, or may lack all or a portion of the leader sequence. Optionally, protein-based therapy may be used in conjunction with administration of a viral-mediated hfH variant. In one embodiment, the fH protein can provide an immediate release form of the hfH to the subject, *e.g.*, detectable plasma levels within 2 hours post-administration, which typically will begin to be cleared from the subject within about 24 hours to about 48 hours, or to about 72 hours, should any lag time in the onset of expression from the viral-mediated delivery system be found to exist. In another embodiment, the hfH variant is further modified to extend its half-life by engineering into the variant at least one glycosylation site is engineered into at least one of the SCRs present in the variant, at least two of the SCRs present in the variant, at least three of the SCRs present in the variant, or more. For example, the glycosylation site may be engineered into one or more of SCR1, SCR2, SCR3, SCR4, SCR19, and/or SCR20. In another embodiment, SCR17 and/or SCR18 are additionally or alternatively glycosylated. In still a further embodiment, SCR4, 17 and 18 are glycoslyated. In certain embodiments, a glycosylation site may be engineered into a linker. However, in such instance, the linker is preferably at least six amino acids in length up to about 18 amino acids in length, *e.g.*, 8 - 18, 10-15, or 12 amino acids. Additionally, or alternatively, the engineered hfH protein variant may be pegylated, *i.e.*, modified with a polyethylene glycol moiety using known techniques [see, *e.g.*, Fee, Conan J.; Van Alstine, James M. (2006). "PEG-proteins: Reaction engineering and separation issues". Chemical Engineering Science 61 (3): 924].

As used herein, a glycosylation site refers to the point of attachment of oligosaccharides to a carbon atom (C-linked), nitrogen atom (N-linked), or oxygen atom (O-linked), or glycation (non-enzymatic attachment of reducing sugars to the nitrogen atom of a protein (*e.g.,* the nitrogen atom of an asparagine (Asn) side chain that is part of an Asn-X-Ser/Thr, wherein X is any amino acid except Pro). In certain embodiments, N-

glycosylation sites are desired. A variety of techniques are known in the art for engineering N-glycosylation sites. *See. e.g.* Y Liu *et al*, Biotech Prog 2009 Sep - Oct; 25(5): 1468-1475; Sala RJ, Griebenos K. Glycoslylation of therapeutic proteins: an effective strategy to optimize efficacy. BioDrugs. 2010 Feb 1; 24(1): 9-21.

Further, an engineered hfH variant as provided herein may be formulated with a suitable carrier and/or excipient for delivery to a subject by any suitable route. In addition to conventional suspension carriers, the carrier may be a liposome or a nanocarrier. Suitable doses of the hfH variant include those which achieve sufficient plasma levels to treat a complement related disorder. Examples of dosages of hfH variants include, but are not limited to, an effective amount within the dosage range of any of about 0.01 μg/kg to about 300 mg/kg, or within about 0.1 μg/kg to about 40 mg/kg, or with about 1 μg/kg to about 20 mg/kg, or within about 1 μg/kg to about 10 mg/kg. For example, when administered intraocularly, the composition may be administered at low microgram ranges, including for example about 0.1 μg/kg or less, about 0.05 μg/kg or less, or 0.01 μg/kg or less. In some embodiments, the amount of hfH variant administered to an individual is about 10 μg to about 500 mg per dose, or about 10 μg to about 50 μg, about 50 μg to about 100 μg, about 100 μg to about 200 μg, about 200 μg to about 300 μg, about 300 μg to about 500 μg, about 500 μg to about 1 mg, about 1 mg to about 10 mg, about 10 mg to about 50 mg, about 50 mg to about 100 mg, about 100 mg to about 200 mg, about 200 mg to about 300 mg, about 300 mg to about 400 mg, or about 400 mg to about 500 mg per dose.

The pharmaceutical compositions may be administered alone. Optionally, the compositions described herein may be administered in combination with other molecules known to have a beneficial effect. For example, useful cofactors include symptom-alleviating cofactors, including antiseptics, antibiotics, antiviral and antifungal agents and analgesics, anti-inflammatories, anesthetics. In another embodiment, where intra-ocular administration is contemplated, molecules helpful for retinal attachment or treatment of damaged retinal tissue may be desired. Examples of useful, cofactors include anti-VEGF agents (such as an antibody against VEGF), basic fibroblast growth factor (bFGF), ciliary neurotrophic factor (CNTF), axokine (a mutein of CNTF), leukemia inhibitory factor (LIF), neutrotrophin 3 (NT-3), neurotrophin-4 (NT-4), nerve

growth factor (NGF), insulin-like growth factor II, prostaglandin E2, 30 kD survival factor, taurine, and vitamin A. Another suitable therapeutic may include an anti-complement antibody, *e.g.*, an anti-complement regulator C3 (*e.g.,* such as is commercially available as Eculizumab).

The compositions described herein (both vector-mediated and protein-based) may be administered to a subject via any route, including, but not limited to, intravenous (*e.g.*, by infusion pumps), intraperitoneal, intraocular, intra-arterial, intrapulmonary, oral, inhalation, intravesicular, intramuscular, intra-tracheal, subcutaneous, intraocular (including, intravitreal, and intra-retinal), intrathecal, transdermal, transpleural, intraarterial, topical, inhalational (*e.g.*, as mists of sprays), mucosal, (such as via nasal mucosa), subcutaneous, transdermal, gastrointestinal, intraarticular, intracistemal, intraventricular, rectal (*i.e.*, via suppository), vaginal (*i.e.*, via pessary), intracranial, intraurethral, intrahepatic, and intratumoral. In some embodiments, the compositions are administered systemically (for example by intravenous injection). In some embodiments, the compositions are administered locally (for example by intraarterial or intraocular injection).

Thus, in a further aspect, use of a pharmaceutical composition in treating a complement related disorder including, *e.g.*, a complement factor H associated disorder such as described herein and other complement related disorders, including, without limitation: tissue damage due to ischemia-reperfusion following acute myocardial infarction, aneurysm, stroke, hemorrhagic shock, crush injury, multiple organ failure, hypovolemic shock intestinal ischemia, spinal cord injury, and traumatic brain injury; inflammatory disorders, *e.g.*, burns, endotoxemia and septic shock, adult respiratory distress syndrome, cardiopulmonary bypass, hemodialysis; anaphylactic shock, severe asthma, angioedema, Crohn's disease, sickle cell anemia, poststreptococcal glomerulonephritis, membranous nephritis, and pancreatitis; transplant rejection, *e.g.*, hyperacute xenograft rejection; pregnancy related diseases such as recurrent fetal loss and pre-eclampsia; adverse drug reactions, *e.g.*, drug allergy, IL-2 induced vascular leakage syndrome and radiographic contrast media allergy; andautoimmune disorders including, but not limited to, myasthenia gravis, Alzheimer's disease, multiple sclerosis, emphysema, obesity, rheumatoid arthritis, systemic lupus erythematosus, multiple

sclerosis, myasthenia gravis, insulin-dependent diabetes mellitus, acute disseminated encephalomyelitis, Addison's disease, antiphospholipid antibody syndrome, autoimmune hepatitis, Crohn's disease, Goodpasture's syndrome, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, idiopathic thrombocytopenic purpura, pemphigus, Sjogren's syndrome, and Takayasu's arteritis, post cardiopulmonary bypass complications; myocardial infarction; ischemia/reperfusion injury; stroke; acute respiratory distress syndrome (ARDS); sepsis; burn injury; inflammation associated with cardiopulmonary bypass and hemodialysis; plasmapheresis; plateletpheresis; leukophereses; extracorporeal; membrane oxygenation (ECMO); heparin-induced extracorporeal LDL precipitation (HELP); radiographic contrast media induced allergic response; transplant rejection.

It is to be noted that the term "a" or "an" refers to one or more. As such, the terms "a" (or "an"), "one or more", and "at least one" are used interchangeably herein.

The words "comprise", "comprises", and "comprising" are to be interpreted inclusively rather than exclusively. The words "consist", "consisting", and its variants, are to be interpreted exclusively, rather than inclusively. While various embodiments in the specification are presented using "comprising" language, under other circumstances, a related embodiment is also intended to be interpreted and described using "consisting of" or "consisting essentially of" language.

As used herein, the term "about" means a variability of 10% from the reference given, unless otherwise specified.

The term "regulation" or variations thereof as used herein refers to the ability of a composition to inhibit one or more components of a biological pathway.

Unless otherwise specified herein, both homozygous subjects and heterozygous subjects are encompassed within the phrase subject having a complement mediated disorder.

A "subject" is a mammal, *e.g.*, a human, mouse, rat, guinea pig, dog, cat, horse, cow, pig, or non-human primate, such as a monkey, chimpanzee, baboon or gorilla.

As used herein, "disease", "disorder", "dysfunction" and "condition" are used interchangeably, to indicate an abnormal state in a subject, unless otherwise specified.

Unless defined otherwise in this specification, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art and by reference to published texts, which provide one skilled in the art with a general guide to many of the terms used in the present application.

The following examples are illustrative only and are not intended to limit the present invention.

EXAMPLES

Engineering and cloning of human factor H truncation variant (hfH1-4.678.19-20):

Truncation variants were generated by inverse PCR method using Phusion high-fidelity DNA polymerase (Cat# M0530S, New England Biolabs) according to manufacturer's protocol. Full-length human complement factor H cDNA pCMV Sport6 used as template for inverse PCR was obtained from Thermo Fisher Scientific (Cat # MHS6278-202800294, clone ID 40148771). PCR primers used for generation of hfH1-4.678.19-20 are listed in Table 1. After PCR fragments were separated on 0.8% agarose gel and extracted by AccuPrep gel extraction kit (Cat# K-3035, Bioneer), 50 ng of gel purified fragment was used for ligation by Rapid DNA ligation Kit (Cat# K-4123, Thermo Fisher Scientific) and transformed into DH5α competent cells (Cat # 1825801, Invitrogen). Positive clones were confirmed either by restriction digestion or by PCR screening using specific primers. Then, the hfH1-4.678.19-20 insert from pCMV Sport6 was released by EcoR I and Not I digestion and gel purified fragment was blunted by End-repair module (Cat# E6050S, New England Biolabs) and purified. This fragment was sub-cloned into the pCBABG vector (which has a chicken beta-actin promoter with CMV enhancer and a partial intron sequence of the same gene, and a rabbit beta-globulin gene polyadenylation signal sequence) at EcoR V site. Positive clones were selected by restriction digestion and PCR methods.

Table1:

hfH Truncation variant Primers:	
hfHdSCR5R SEQ ID NO:49	TGA TTT TTC TTC ACA TGA AGG CAA CGG
hfHdSCR5F SEQ ID NO:50	ACC TTG AAA CCT TGT GAT TAT CCA GAC A
hfHdSCR9-18R SEQ ID NO: 51	AGA TTT AAT GCA CGT GGG TTG AGC
hfHdSCR9-18F SEQ ID NO: 52	AAA GAT TCT ACA GGA AAA TGT GGG CC

Engineering and cloning of human factor H truncation variant hfH1-4.678.17-20:

Truncation variants were generated by inverse PCR method using *Phusion* high-fidelity DNA polymerase (Cat# M0530S, New England Biolabs) according to manufacturer's protocol. Full length human complement factor H cDNA pCMV Sport6 (used as template for inverse PCR) was obtained from Thermo Fisher Scientific (Cat # MHS6278-202800294, clone ID 40148771). PCR primers used for generation of hfH1-4.678.17-20 are listed in Table 1. After PCR fragments were separated on 0.8% agarose gel and extracted by AccuPrep gel extraction kit (Cat# K-3035, Bioneer), 50 ng of gel purified fragment was used for ligation by Rapid DNA ligation Kit (Cat# K-4123, Thermo Fisher Scientific) and transformed into DH5α competent cells (Cat # 1825801, Invitrogen). Positive clones were confirmed either by restriction digestion or by PCR screening using specific primers. Then, the engineered hfH1-4.678.17-20 variant in pCMV Sport6 was sub-cloned into pCBABG vector at EcoRI site by infusion cloning method (Clontech Cat# 638909). Primers for truncation protein preparation and cloning into expression vector were in Table 2.

Table 2:

hfH Truncation variant Primers:	
hfHdSCR5R SEQ ID NO: 49	TGA TTT TTC TTC ACA TGA AGG CAA CGG

hfH Truncation variant Primers:	
hfHdSCR5F SEQ ID NO: 50	ACC TTG AAA CCT TGT GAT TAT CCA GAC A
hfHdSCR9-16R SEQ ID NO: 53	AGA TTT AAT GCA CGT GGG TTG AGC
hfHdSCR9-16F SEQ ID NO: 54	ATAAAAACAGATTGTCTCAGTTTACCTAGCT
pCBAGhfH-ORF F SEQ ID NO: 55	TTTTGGCAAAGAATTGGACGTTGTGAACAGAGTT
pCBAGhfH-ORF R SEQ ID NO: 56	CCTGAGGAGTGAATTCTATCTTTTTGCACAAGTTGG

Expression and purification of recombinant hfH1-4.678.19-20 protein:

Positive clones (hfH1-4.678.19-20 in pCBARBG vector) were transfected into HEK cells to assess the stability and functional activity of hfH1-4.678.19-20 protein. About 80% confluent HEK cells in a 6-well plate (Falcon, Cat# 353046) were transfected with hfH1-4.678.19-20 cDNA in pCBARBG using Lipofectamine 2000 (Cat # 11668019, Invitrogen) according to manufacturer's instructions. Protein expression was confirmed by western blotting using goat anti-human factor H IgG (Cat # A237, Complement tech). For large scale protein expression, 80% confluent HEK cells in 150cm dishes (Falcon, Cat# 353025) were transfected with endotoxin free hfH1-4.678.19-20 cDNA in pCBARBG plasmid with PEI (Cat# 23966, Polysciences) according to manufacturer's instructions. Two days post-transfection, supernatant was collected from the plates and filtered through 0.2 μm filter and loaded onto a PBS-equilibrated, Ox-23 (mouse anti-human fH mAb specific for SCR 2/3, cat# 10402-1VL, Sigma) sepharose affinity column. After washing with PBS containing 500mM NaCl with 25 column volumes, bound hfH1-4.678.19-20 was eluted with 100mM Glycine HCl pH2.7 and eluted fractions (2 ml per fraction) were neutralized with 200ul of 1.5M Tris-HCl pH 8.5. Eluted protein purity was checked by SDS-PAGE and pure fraction were pooled and dialyzed with PBS with 2 changes overnight.

Engineering and cloning of mouse factor H truncation variant (mfH1-4.678.19-20):

Truncation variants were generated by inverse PCR method using Phusion high-fidelity DNA polymerase according to manufacturer protocol. Full-length mouse complement factor H cDNA in pBluescript SK(-) used as template for inverse PCR was kindly provided by Dr M. Nonaka (University of Tokyo, Japan, Nucleotide 110-4361 of NCBI NM 009888.3). All PCR primers used for generation of mfH1-4.678.19-20 variant are listed in Table 3. After PCR fragments were separated on 0.8% agarose gel and extracted by AccuPrep gel extraction kit, 50 ng of gel purified fragment was used for ligation by Rapid DNA ligation Kit and transformed into DH5α competent cells. Positive clones were confirmed either by restriction digestion or by PCR screening using specific primers. Then, mfH1-4.678.-19-20 insert from pBluescript SK(-) was released by Sma I and EcoR V digestion and gel purified. This fragment was sub-cloned into pCBARBG vector at EcoR V site. Positive clones were selected by restriction digestion and PCR methods.

Table 3:

mfH Truncation variant Primers:	
dSCR5R SEQ ID NO: 57	TCTCTTTTCTTCACAGAAAGGCTGAGAACTCC
dSCR5F SEQ ID NO: 58	ACC TTG AAA CCA TGT GAA TTT CCA CAA TTC
dSCR9-18F SEQ ID NO: 59	CGA GAC TCA ACA GGG AAA TGT GG
dSCR9-18R SEQ ID NO: 60	AGA CTT AAT GCA TGA GGG TTG AGG T

Expression of recombinant mfH1-4.678.19-20 protein:

Positive clones (mfH1-4.678.19-20 in pCBARBG vector) were transfected into Hepa1C1C7 cells (mouse hepatoma cell line, ATCC® CRL-2026) to assess stability and functional activity of mfH1-4.678.19-20 protein. About 80% confluent cells in a 6-well

plate were transfected with mfH1-4.678.19-20 cDNA using Lipofectamine 2000 according to manufacturer's instructions. Protein expression was confirmed by western blotting using rabbit anti-mouse fH IgG (Ref #1). Blots were visualized using Pierce ECL plus Western Blotting substrate (Cat# 80196, Thermo Fisher Scientific).

Generation of AAV transfer plasmid and virus:

mfH1-4.678.19-20 or hfH1-4.678.19-20 expression cassette from pCBARBG vector was released by Hinc II and Pst I digestion and gel purified fragment was blunted with the End Repair Module (cat# E6050S, NEB) and ligated into Nhe I- and Xho I-digested and blunted pAAV TBG.PI.EGFP.WPRE.BGH vector (Cat# PL-C-PV0146) from the University of Pennsylvania Vector Core http://www.med.upenn.edu/gtp/vectorcore/production.shtml). Positive clones were screened by Sma I digestion.

pCBABG with hfH1-4.678.17-20 vector was modified into AAV transfer plasmid by inserting the ITRs (inverted terminal repeats) at 5' end (SEQ ID NO: 61: ctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgtcgggcgacctttggtcgcccggcctcagtgagc gagcgagcgcgcagagagg-gagtggccaactcc-atcactaggggttccttgtagttaat, at HincII site) and 3'end (SEQ ID NO: 62: attaactacaaggaacccctagtgatggagttggccactccctctctgcgcgctcgctcgctcactgaggccgggcgaccaaa ggtcgcccgacgcccgggctttgcccgggcggcctcagtgagcgagcgagcgcgcag, at Pst I site) of the expression cassette by using Infusion cloning method. Primers were used to amplify the AAV ITRs from the pENN.AAV.TBG.PI.RBG vector used as template listed in Table 4. The pENN.AAV.TBG.PI.RBG vector was obtained from the University of Pennsylvania Vector Core http://www.med.upenn.edu/gtp/vectorcore/ production.shtml) (Cat# PL-C-PV1015).

Table 4

ITR insertion primers	
Hinc II 5'ITR F SEQ ID NO: 63	AAGTGCCACCTGGTCGACGCTGCGCGCTCGCTCGCT
Hinc II 5'ITR R	TCAATAATCAATGTCGACATTAACTACAAGGAACCCCT

ITR insertion primers	
SEQ ID NO: 64	
Pst I 3'ITR F SEQ ID NO: 65	GAAGATCCCTCGACCTGCAGATTAACTACAAGGAACCCCT
Pst I 3'ITR R SEQ ID NO:66	ACGCCAAGCTTGGGCTGCAGCTGCGCGCTCGCTCGCTC

Super-coiled endotoxin-free AAV plasmid was prepared by Endo free plasmid kit (cat# 12362, Qiagen), and was used for AAV virus production by the University of Pennsylvania Vector Core or the University of Massachusetts Gene Therapy Center Vector Core. The packaging, purification, and titer determination of AAV encoding mfH1-4.678.19-20, hfH1-4.678.19-20 or hfH1-4.678.17-20 was accomplished by using standard procedures as described (http://www.med.upenn.edu/gtp/vectorcore/production.shtml).

Therapeutic efficacy of hfH1-4.678.19-20 and hfH1-4.678.17-20 AAV in $fH^{m/m}$ mice:

The generation of $fH^{m/m}$ mice which developed C3 glomerulopathy has been described previously in the paper by Lesher et al (2013) "Combination of factor H mutation and properdin deficiency causes severe C3 glomerulonephritis", J Am Soc Nephrol. 2013 Jan; 24(1):53-65. Epub 2012 Nov 30. To test the expression levels, duration and therapeutic efficacy of hfH1-4.678.19-20 and hfH1-4.678.17-20 in treating C3 glomerulopathy, 10-12 weeks old $fH^{m/m}$ mice were injected with 3×10^{12} gene copies/mouse (for hfH1-4.678.19-20) or 1×10^{11} - 1×10^{12} gene copies/mouse (for hfH1-4.678.17-20) by retro-orbital route. In separate groups of mice, a control AAV vector (pAAV.TBG.NULL.rBG) was used as a control. It is known from previous studies that natural human fH is functionally active in inhibiting alternative pathway (AP) complement activation in mice (Fakhouri, F., et al, Kidney International (2010) 78, 279–286; published online 5 May 2010). Blood was collected via retro-orbital bleed prior to injection and at 1 week after injection (for hfH1-4.678.19-20) or 1, 2 weeks, 1, 2, 3 months after injection (for hfH1-4.678.17-20). The $fH^{m/m}$ mice develop spontaneous

C3 glomerulopathy characterized by uncontrolled plasma AP complement activation, leading to C3, factor B (fB) and C5 consumption and prominent glomerular deposition of C3 and C5b-9 (Lesher et al 2013). If hfH1-4.678.19-20 or hfH1-4.678.17-20 is functionally active in $fH^{m/m}$ mice, one would expect a reduction in C3 and fB consumption. Therefore, as a readout for the therapeutic efficacy of hfH1-4.678.19-20 and hfH1-4.678.17-20, we examined the levels of plasma C3 and fB by western blot before and after AAV injection into $fH^{m/m}$ mice. Mouse plasma (1µl) was diluted with sample buffer and boiled before loading onto 4-20% gradient SDS-PAGE gels under reducing conditions. Samples were then transferred to PVDF membrane and probed with appropriate antibodies. For the detection of C3 and fB, HRP-conjugated goat anti-mouse C3 Ab (1:4000, MP Biomedicals Cat # 0855557) or affinity-purified goat anti-human fB Ab (cross-reacts with mouse fB; 1:2500, cat#A235, Complement Technology) were used as primary antibodies, followed by HRP-conjugated rabbit anti-goat IgG (1:4000, Cat # 1721034, Bio-Rad). Blots were visualized using Pierce ECL Plus Western Blotting substrate.

Detection of hfH1-4.678.19-20 or hfH1-4.678.17-20 protein in mouse blood:

To detect the presence of hfH1-4.678.19-20 or hfH1-4.678.17-20 in AAV-treated $fH^{m/m}$ mice, an ELISA method was developed and used. Briefly, 96-well plates (MaxiSorp) were pre-coated with 4 µg/ml of anti-human factor-H mAb (OX-23) at RT for 2hr. Un-occupied binding sites on the plates were blocked using 1% bovine serum albumin (BSA) in PBS at RT for 1 h. Serially diluted mouse plasma samples in blocking buffer containing 10mM EDTA were added to the wells and incubated at RT for 1 h, followed by 2ug/ml of biotin-labeled anti-hfH mAb (clone L20/3, specific for SCR19 of human factor-H, Cat# 518504, Bio-Legend) and incubated at RT for 1 h. After washing, plates were then incubated with Avidin-HRP (1/1000, Cat 554058, BD Biosciences) at RT for 1 h, and developed using the TMB substrate reagent (Cat 51-2606KC and BD Cat 51-2607KC, BD Biosciences).

Therapeutic efficacy of mfH1-4.678.19-20 delivered by AAV in $fH^{m/m}$ or $fH^{m/m}P^{-/-}$ mice:

To test the therapeutic efficacy of mfH1-4.678.19-20 as a surrogate for hfH1-4.678.19-20, $fH^{m/m}$ mice and $fH^{m/m}P^{-/-}$ mice were infected with AAV vector containing the coding sequences for mfH1-4.678.19-20. As previously described by Lesher et al (Lesher et al, 2013, cited above), while $fH^{m/m}$ mice developed non-lethal C3 glomerulopathy with C3 and fB consumption, the double mutant $fH^{m/m}P^{-/-}$ mice ($fH^{m/m}$ mice that were rendered deficient in properdin) developed an exacerbated and lethal form of C3 glomerulopathy and died by 10-12 week old (Lesher et al 2013). Therefore, the $fH^{m/m}P^{-/-}$ mice would also allow us to use mortality as another readout for the therapeutic efficacy of mfH1-4.678.19-20 AAV. 7-week old $fH^{m/m}$ or $fH^{m/m}P^{-/-}$ mice were injected with either control AAV (pAAV.TBG.NULL.rBG) or mfH1-4.678.19-20 AAV at 3 x 10^{12} gene copies/mouse by retro-orbital route. Blood was collected via retro-orbital bleeding prior to injection at various time points starting at 1 week after injection. To assess plasma C3 and fB levels, mouse plasma (1µl) was diluted with sample buffer and boiled before loading onto 4-20% gradient SDS-PAGE gels under reducing conditions. Samples were then transferred to PVDF membrane and probed with appropriate antibodies. For C3 and fB, HRP-conjugated goat anti-mouse C3 Ab or affinity-purified goat anti-human fB Ab (cross reacts with mouse fB) were used as primary antibodies, followed by detection with HRP-conjugated rabbit-anti goat IgG. In some cases, the treated mice were followed for 6 or 10 months to observe the efficacy of mfH1-4.678.19-20 AAV in preventing death and/or AP complement activation using plasma C3 and fB levels as readouts.

Dosage determination of mfH1-4.678.19-20 AAV in $fH^{m/m}$ mice:

In experiments aimed at titrating the amount of mfH1-4.678.19-20 AAV copies needed to achieve therapeutic efficacy, 10-12 weeks old $fH^{m/m}$ mice (Lesher, 2013) were injected with 1 x 10^{12}, 3 x 10^{11} or 1 x 10^{11} gene copies/mouse of AAV by retro orbital route. Blood was collected via retro-orbital bleeds prior to injection and at indicated time points (1 week and 1 month after injection). Mouse plasma (1µl) was diluted with sample buffer and boiled before loading onto 4-20% gradient SDS-PAGE gels under reducing

conditions. Samples were then transferred to PVDF membrane and probed with appropriate antibodies. For the detection of C3 and fB, HRP-conjugated goat anti-mouse C3 Ab (1:4000, Cat # 0855557, MP Biomedicals) or affinity-purified goat anti-human fB Ab (cross-reacts with mouse fB; 1:2500, cat#A235, Complement Technology, Inc.) were used as primary antibodies, followed by detection with HRP-conjugated rabbit anti-goat IgG (1:4000, Cat # 1721034, Bio-Rad). Blots were visualized using Pierce ECL Plus Western Blotting substrate.

Detection of mfH1-4.678.19-20 protein in mouse blood by ELISA:

To detect the presence of mfH1-4.678.19-20 protein in the mouse blood, an ELISA assay was developed and used. Briefly, 96-well plates were pre-coated with 2 µg/ml of mouse anti-mouse fH SCR19-20 mAb (clone-12, generated in-house by immunizing $fH^{m/m}$ mice with recombinant mouse fH SCR19-20 (Barata, L., et al, J. Immunol 190(6): 2886-95 (2013)) at 37°C for 1-2 hr at room temperature. Un-occupied binding sites on the plates were blocked with 1% BSA in PBS at RT for 1 hr. Serially diluted mouse plasma samples in blocking buffer containing 10 mM EDTA were added to wells and incubated at RT for 1 hr, followed by biotin-labeled rabbit anti-mouse fH Ab (Lesher et al, 2013) at RT for 1 hr. Plates were incubated with Avidin-HRP at RT for 1 hr, then developed using the TMB substrate reagent.

Detection of mfH1-4.678.19-20 protein in mouse plasma by western blotting:

To detect the presence of mfH1-4.678.19-20 protein in the mouse blood by western blot, 10 µl of mouse plasma was diluted with 90ul of PBS containing 10mM EDTA and incubated with anti-mouse fH mAb (clone-12)-coupled Sepharose® beads for 30 min at room temperature. After washing 2 times with PBS containing 500mM NaCl, the Sepharose® beads were boiled with SDS-PAGE sample buffer for 5 min and run on SDS-PAGE. Samples were then transferred to PVDF membrane and mfH1-4.678.19-20 protein was detected by BSA pre-absorbed rabbit anti mouse fH 19-20 Ab (Lesher et al, 2013). Blots were visualized using Pierce ECL Plus Western Blotting substrate.

Immuno-fluorescence staining of C3 in kidney:

Kidneys from control AAV- or mfH1-4.678.19-20 AAV-treated $fH^{m/m}$ or $fH^{m/m}P^{-/-}$ mice were snap-frozen in OCT medium and stored at -80°C. For immunofluorescence studies, 4μm sections were cut and used for staining. For C3 staining, FITC-conjugated goat anti-mouse C3 Ab was used (1:500, Cat # 855500, MP Biomedicals) and the experiment was performed as described (Lesher et al 2013).

Mouse survival analysis:

The following Table provides a summary of survival data of $fH^{m/m}P^{-/-}$ mice treated with control AAV8 vector or AAV8-mfH1-4.678.19-20 vector. All 8 $fH^{m/m}P^{-/-}$ mice treated with control AAV8 vector died within 2-3 weeks of treatment, whereas 7 out of 9 $fH^{m/m}P^{-/-}$ mice treated with the AAV8-mfH1-4.678.19-20 vector were rescued from lethal C3 glomerulopathy. All mice were injected with 3×10^{12} gene copies/mouse of the respective AAV virus through retro-orbital I.V. routes. Survival of control AAV- or mfH1-4.678.19-20 AAV-treated $fH^{m/m}P^{-/-}$ mice was recorded after AAV treatment for 10 months. Data were categorized as being censored (euthanized) or natural death and analyzed by GraphPad Prism (La Jolla,CA).

AAV vector	Number of mice treated	Note
AAV8-mfH1-4.678.19-20	9 mice	4- healthy at 9 month after gene therapy (continuing) 2- healthy at 6 month after gene therapy (sacrificed at 6 month) 1-healthy at 5 month after gene therapy (continuing) 1 Moribund at 3 month after gene therapy 1- Moribund at 2 weeks after gene therapy
Con AAV8	8 Mice	All died 2-3 W post injection

Heparin-binding assay:

To test the Heparin-binding activity of hfH1-4.678.19-20 and mfH1-4.678.19-20 proteins, 96-well plates were pre-coated with 100 μg of Heparin (Sigma, H3393) in bicarbonate buffer (pH9.6) at 37°C for 1hr. The unoccupied binding sites on the plates were blocked with 1% BSA in PBS at RT for 1 hr. Different amounts of hfH1-4.678.19-20 or mfH1-4.678.19-20 protein were added and incubated at RT for 1 hr, followed by 2 μg/ml of mouse anti-human fH mAb (OX-23) at RT for 1 hr. Plates were incubated with HRP-conjugated rabbit anti-mouse IgG (1/4000, Cat #A9044, Sigma) at RT for 1 hr, then developed using the TMB substrate reagent.

C3b-binding assay:

To test the C3b-binding activity of hfH1-4.678.19-20 and mfH1-4.678.19-20 proteins, 96-well plates were pre-coated with 2 μg/ml human C3b (Cat # A114, CompTech) at 37°C for 1hr. The unoccupied binding sites on the plates were blocked with 1% BSA in PBS at RT for 1 h. Different amounts of hfH1-4.678.19-20 or mfH1-4.678.19-20 protein were added and incubated at RT for 1 h, followed by 2ug/ml of mouse anti-human fH mAb (OX-23) at RT for 1 hr. Plates were incubated with HRP-conjugated rabbit anti-mouse IgG at RT for 1 hr, then developed using the TMB substrate reagent.

Assay of fluid-phase cofactor activity of fH protein in factor I-mediated C3b cleavage:

To assess the fluid phase cofactor activity of hfH1-4.678.19-20 and mfH1-4.678.19-20 proteins in factor I-mediated cleavage of C3b, 0.5 or 0.25μg of purified hfH1-4.678.19-20 or mfH1-4.678.19-20 protein was mixed with 2μg of human C3b in 15 μl PBS, and 1μg of human factor I (Cat# A138, CompTech) was subsequently added and incubated at 37°C for 15 minutes. Reaction was stopped by adding 5x reducing SDS-PAGE sample buffer. Proteolysis of C3b was determined by analyzing the cleavage of the α chain and the generation of the α41 and α39 fragments using 4-20% Gradient SDS-PAGE gels under reducing conditions, followed by western blot detection using HRP-

conjugated goat anti-human C3 IgG (1/4000, Cat# 855237, MP biomedicals). Blots were visualized using Pierce ECL Plus Western Blotting substrate.

Assessment of therapeutic efficacy of mfH1-4.678.19-20 in preventing AP complement activation caused by membrane complement regulator defects:

To determine if fH1-4.678.19-20 AAV treatment may also be effective in preventing AP complement activation caused by defects in membrane complement regulators, mfH1-4.678.19-20 was tested in a strain of mouse that is deficient in two membrane complement regulators DAF and Crry (DAF/Crry double mutant mice). The generation of DAF/Crry double mutant mice ($DAF^{-/-}$-$Crry^{flox/flox}$-Tie-$2Cre^{+}$) was previously described with a phenotype of secondary complement deficiency due to excessive AP complement activation (Barata et al, 2013). Like $fH^{m/m}$ mice, there was C3 and fB consumption in the DAF/Crry double mutant mice (Barata et al, 2013). DAF/Crry double mutant mice (10-week old) were injected with mfH1-4.678.19-20 AAV at 3 x 10^{12} gene copies/mouse by retro orbital route. Blood was collected via retro-orbital bleeds prior to injection and at 1 week after injection. Therapeutic efficacy was assessed by measuring plasma C3 and fB levels before and after mfH1-4.678.19-20 AAV treatment using western blot analysis. For western blot, mouse plasma (1 μl) was diluted with sample buffer and boiled before loading onto 4-20% gradient SDS-PAGE gels under reducing conditions. Samples were then transferred to PVDF membrane and probed with appropriate antibodies. For the detection of mouse C3 and fB, HRP-conjugated goat anti-mouse C3 Ab (1:4000, Cat # 0855557, MP Biomedicals) or affinity-purified goat anti-human fB Ab (Cat # A235, CompTech, Texas, across reacts with mouse fB) were used as primary antibodies, followed by detection with HRP-conjugated rabbit anti-goat IgG. Blots were visualized using Pierce ECL Plus Western Blotting substrate.

Generation of aHUS mouse model:

To create a murine aHUS model for testing the therapeutic efficacy of AAV-mediated fH gene therapy, a mutant mouse strain carrying a fH point mutation in SCR20 corresponding to human fH W1183R mutation found in aHUS patients was created by

homologous recombination-based gene targeting technique (Lesher et al, 2013; Dunkelberger, et al, J Immunol. 2012 Apr 15; 188(8): 4032–4042; Takashi et al, Blood. 2009 Mar 19; 113(12): 2684–2694; Kimura Y1, et al., Blood. 2008 Jan 15;111(2):732-40. Epub 2007 Oct 4; Kimura Y1, et al, J Clin Invest. 2010 Oct;120(10):3545-54). For this experiment, fH gene fragments were amplified from C57BL/6 mouse genomic DNA by using the Expand Long Template PCR system (Roche, Indianapolis, IN) in order to construct the gene targeting vector. The long arm of targeting vector was comprised of a 6 kb fragment containing the 21th exon and flanking intronic sequences of the mouse fH gene. It was amplified by PCR using the following primers: SEQ ID NO: 67: 5'-gcggccgccctatccattagtgagtgtgg-3' and SEQ ID NO: 68: 5'-ctcgaggacagcgatgtaagaacaatc-3'. The PCR product was ligated into PCR 2.1 vector (Invitrogen) and the insert was then released from PCR2.1 vector by with Not I and XhoI restriction digestion, purified and sub-cloned into the pND1 vector upstream of the NEO cassette. The use of pND1 vector has been described in previous publications of gene targeting experiments (Lesher et al (2013); Dunkelberger et al, 2012; Miwa et al, 2009; Kimura et al, 2008; Kimura et al 2012) and this vector contains neomycin (NEO) and diphtheria toxin (DT) cassettes for positive and negative selection, respectively (Lesher et al (2013); Dunkelberger et al, 2012; Miwa et al, 2009; Kimura et al, 2008; Kimura et al 2012). The pND1 vector also contains a loxP site and two flippase recognition target (FRT) sites flanking the NEO cassette for potential removal of NEO by FLPe recombinase (Rodríguez CI, et al, Nat Genet. 2000 Jun;25(2):139-40.).

The short arm sequence was comprised of a 3.85 kb fragment containing the 22th exon encoding SCR20 and the flanking intronic sequences of the mouse fH gene. This sequence was PCR-amplified using the following primers: SEQ ID NO: 69: 5'-ggtaccaagcttattgaccagctacagacagta-3' and SEQ ID NO: 70: 5'-ggtaccctcactcaggtgtattactc-3'. The PCR product was cloned into PCR 2.1 vector and subsequently a tryptophan (W) to arginine (R) mutation at position 1206 corresponding to W1183R mutation of human fH in SCR20 was made by site-directed mutagenesis using the Stratagene QuickChange Site-Directed Mutagenesis kit (Agilent Technologies, CA) with the following two primers, SEQ ID NO: 71: 5'-GGAATCACACAATATAATTCTCAAAAGGAGACACACTG-3' and SEQ ID NO:

72: 5'-CAGTGTGTCTCCTTTTGAGAATTATATTGTGTGATTCC-3'. After W to R mutation was confirmed, the short arm fragment was released from PCR2.1 by Kpn I digestion and sub-cloned into the pND1 vector downstream of the NEO cassette at the same restriction site. The targeting vector was then linearized by Not I digestion and transfected into C57BL/6 embryonic stem (ES) cells (EmbroMAX Embryonic stem cell line-strain C57BL/6, Cat # CMTI-2, Millipore) by electroporation-method. Transfected ES cells were subjected to G418 selection starting from 48 hours after electroporation. ES cells with homologous recombination were screened by Southern blot analysis of genomic DNA after *Hind*III digestion with a 480bp 3′ probe amplified using SEQ ID NO: 73: 5'-ATAGCATGTGCCAGGAGACAC-3' and SEQ ID NO: 83: 5'-AGTGTTGACTCGTGGAGACCA-3' as primers. Wild-type allele produced a 12.5kb fragment, whereas the targeted allele produced a 10.2kb fragment. Correctly targeted ES cells ($fH^{W1201R\ (Neo\text{-}positive)/+}$) were injected into 3.5-day post-coital C57BL/6J blastocysts to generate chimeras at the University of Pennsylvania School of Medicine Transgenic Core Facility. The resultant chimeras yielded germ line transmission, as assessed by a combination of coat color and PCR screening for the detection of NEO using the following two primers: Neo-4 primer: SEQ ID NO: 74: 5-CTTGGGTGGAGAGGCTATTC-3' and SEQ ID NO: 75: Neo-5 primer: 5'-AGGTGAGATGACAGGAGATC-3'. The neomycin-resistance cassette (NEO) in the targeting vector was flanked by 2 flippase (FLP) recombinase target (FRT) sites to allow its subsequent removal by FLP recombinase. Heterozygous FH-targeted mice ($fH^{W1206R\ (Neo\text{-}positive)/+}$) were crossed with FLPe transgenic mice (expressing the enhanced version of FLP, on C57BL/6 genetic background) to remove the NEO from the fH allele and generate a heterozygous fH mutant mouse without the NEO gene cassette ($fH^{W1206R/+}$). $fH^{W1206R/+}$ mice were intercrossed to generate $fH^{W1206R/W1206R}$ homozygous mice on C57BL/6 genetic background. For genotyping, the following primers were used for detection of wild-type and mutated fH alleles by PCR: WR1 (FH-specific) SEQ ID NO: 76: 5'- GATATGGTCAATTTAGGGAAAGT, SEQ ID NO: 77: Neo7 (NEO-specific) 5'-GGGTGGGATTAGATAAATGCC -3' and SEQ ID NO: 78: WR4 (FH-specific) 5'-TACTGTCTGTAGCTGGTCAAT 3'.

The following table summarizes the treatment outcome of $fH^{W1206R/W1206R}$ mice receiving control AAV or AAV8-mfH1-4.678.19-20 vector at 3 x 10^{11} GC/mouse.

AAV vector	Number of mice treated	Outcome
AAV8-mfH1-4.678.19-20 (3x 10^{11} GC/mouse)	3 mice	• All 3 mice are alive and healthy as of date (2 months after gene therapy) • All have normal platelet counts
Con AAV8 (3x 10^{11} GC/mouse)	2 Mice	• 1 died after 4 weeks of treatment • The remaining mouse is alive but has low platelet count

Homozygous $fH^{W1206R/W1206R}$ mice failed to thrive with significantly lower bodyweights as evidenced at 4-6 weeks of age and a near 50% mortality rate by 30 weeks. All $fH^{W1206R/W1206R}$ mice showed one or more of the characteristic features of aHUS, i.e. renal injury (elevated blood urea nitrogen levels and/or histological signs of thrombotic microangiopathy in glomeruli), thrombocytopenia and anemia. About one third of $fH^{W1206R/W1206R}$ mice also developed severe neurological symptoms indicative of stroke. In addition to thrombotic microangiopathy in the kidney glomeruli, numerous large vessel thrombi in multiple organs (liver, lung, spleen, kidney, brain and eye) were present in $fH^{W1206R/W1206R}$ mice.

As of the timepoints reported above, all 3 $fH^{W1206R/W1206R}$ mice treated with AAV8-mfH1-4.678.19-20 were alive and healthy with normalized platelet counts, whereas 1 of 2 $fH^{W1206R/W1206R}$ mice treated with control AAV vector died (at 4 weeks after treatment) and remaining mouse was displaying symptoms of aHUS including thrombocytopenia.

Therapeutic efficacy of mfH1-4.678.19-20 delivered by AAV in $fH^{W1206R/W1206R}$ mice:

To test the therapeutic efficacy of mfH1-4.678.19-20 as a surrogate for hfH1-4.678.19-20, we injected 4-week old homozygous $fH^{W1206R/W1206R}$ mice with 3 x

10^{11}gene copies/mouse by retro-orbital route. If mfH1-4.678.19-20 is functionally active in $fH^{W1206R/W1206R}$ mice, one would expect a reduction in thrombocytopenia and renal injury. Therefore, as readouts for the therapeutic efficacy of mfH1-4.678.19-20, we counted the number of platelet and measured the level of serum blood urea nitrogen. Since $fH^{W1206R/W1206R}$ mice failed to thrive with significantly lower bodyweights evident at 4-6 weeks of age and a near 50% mortality rate by 30 weeks. The $fH^{W1206R/W1206R}$ mice would also allow us to use mortality as another readout for the therapeutic efficacy of mfH1-4.678.19-20 AAV.

Platelet counts in control AAV- and mfH1-4.678.19-20 AAV-treated $fH^{W1206R/W1206R}$ mice

To determine the platelet counts in control AAV- and mfH1-4.678.19-20 AAV-treated $fH^{W1206R/W1206R}$ mice, blood was collected with EDTA (final concentration: 0.02M) via retro-orbital bleeds prior to injection and at various time points starting at 1 month after injection and analyzed on the Sysmex XT-2000iV Automated Hematology Analyzer at the CTRC Translational Core Laboratory at the Children's Hospital of Philadelphia (https://ctrc.research.chop.edu/services-facilities/translational-core-laboratory-tcl/hematology).

Blood urea nitrogen (BUN) measurement in control AAV- and mfH1-4.678.19-20 AAV-treated $fH^{W1206R/W1206R}$ mice:

To measure the serum level of blood urea nitrogen, blood samples were collected via retro-orbital bleeds prior to injection and at various time points starting at 1 month after injection. Serum BUN levels were measured using urea nitrogen reagents (Sigma-Aldrich) by following the manufacturer's instructions.

Histological examination of kidney and other organs of $fH^{W1206R/W1206R}$ mice:

Paired kidneys and other organs were collected from $fH^{W1206R/W1206R}$ mice. One was fixed in formalin solution overnight and processed for paraffin embedding, and the other was snap-frozen in OCT compound (Sakura Finetek). Kidneys and other organs were evaluated histologically for signs of aHUS/ thrombotic microangiopathy using light microscopy and immunohistochemistry including immunofluorescence and

immunoperoxidase.

(Sequence Listing Free Text)

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42	<223> hfH1-4.678.19-20 protein
43	<223> murine fH1-4.678.19-20
44	<223> mouse factor H truncation construct mFH1-4.678.19-20
45	<223> engineered fH SCR1-4, 6-8, 17-20
46	<223> Synthetic Construct
47	<223> hfH1-4.678.17-20 containing leader and 5' UTR
48	<223> hFH 1-4.678.17-20
49	<223> hfHdSCR5R truncation variant primer
50	<223> hfHdSCR5F truncation primer

51	<223> hfHdSCR9-18R truncation variant primer
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53	<223> hfHdSCR9-16R truncation variant primer
54	<223> hfHdSCR9-16F truncation variant primer
55	<223> pCBAGhfH-ORF F truncation variant primer
56	<223> pCBAghfH-ORF R primer
57	<223> dSCR5R
58	<223> dSCR5F truncation varient primer
59	<223> dSCR9-18F truncation varient primer
60	<223> dSCR9-18R truncation primer
61	<223> AAV 5' ITR
62	<223> AAV 3' ITR
63	<223> Hinc II 5'ITR F insertion primer
64	<223> Hinc II 5'ITR R insertion primer
65	<223> Pst I 3'ITR F insertion primer
66	<223> Pst I 3'ITR R insertion primer
67	<223> mFH primer 21st exon + intron
68	<223> R primer mFH 21st exon + intron
69	<223> F primer mFH SCR20 (exon 22)
70	<223> R primer mFH SCR20 (exon 22)
71	<223> F primer W1183R mutation hFH
72	<223> R primer W1183R mutation
73	<223> F Primer for 480 bp 3' probe
74	<223> Neo-4 primer
75	<223> Neo-5 primer
76	<223> mfH1-4.678.19-20
77	<223> NEO-specific
78	<223> WR4 (FH-specific)
81	<223> mfH1-4.678.19-20

All publications cited in this specification are incorporated herein by reference. US Provisional Application No. 62/232,008 filed September 24, 2015, is also incprorated by reference. Similarly, the SEQ ID NOs which are referenced herein and which appear in the appended Sequence Listing are incorporated by reference. While the invention has been described with reference to particular embodiments, it will be appreciated that modifications can be made without departing from the spirit of the invention. Such modifications are intended to fall within the scope of the appended claims.

CLAIMS:

1. A recombinant vector having packaged therein an expression cassette comprising an engineered human complement regulator factor H (hfH) gene operably linked to expression control sequences which direct expression thereof, wherein said hfH gene encodes a soluble hfH protein variant that retains complement regulatory function, wherein said fH variant comprises:

short consensus repeat (SCR) 1, 2, 3, 4, 19, 20 and one or more of SCR7, 17 and/or 18, wherein following administration of the vector to a subject and expression, detectable plasma levels of the hfH variant are present in the subject for at least a week.

2. The recombinant vector according to claim 1, wherein the hfH variant further comprises additional hfH SCR consisting of SCR5, SCR6, SCR8, SCR16, or combinations thereof.

3. The recombinant vector according to claim 1 or 2, wherein the fH gene lacks coding sequences for at least SCR5, SCR9, SCR10, SCR11, SCR12, SCR13, SCR14, and/or SCR15.

4. The recombinant vector according to claim 1, wherein the fH variant comprises at least one glycosylation site in one or more of the SCRs.

5. The recombinant vector according to claim 1, wherein the hfH variant comprises a combination of SCR domains selected from one or more of:

(a) SCR1, 2, 3, 4, 7, and 19-20;

(b) SCR1- 4, 6, 7, and 19-20 ;

(c) SCR1-4, 7, 8, and 19-20;

(d) SCR1-4, 6, 7, 8, and 19-20;

(e) SCR1-4, 17, 19-20;

(f) SCR1-4, and18-20;

(g) SCR1-4, and 17-20;

(h) SCR1-4, 7, and 18-20;

(i) SCR1-4, 6, 7, and 18-20;

(j) SCR1-4, 7, 8, and 18-20;

(k) SCR1-4, 6-8, and 18-20,

(l) SCR1-4, 7, and 17-20;

(m) SCR1-4, 6, 7, and 17-20;

(n) SCR1-4, 7, 8, and 17-20; or

(o) SCR1-4, 6-8, and 17-20.

6. The recombinant vector according to claim 5, wherein the expressed hfH variant has the amino acid sequence of Figure 4 [SEQ ID NO:43].

7. The recombinant vector according to any one of claims 1 to 6, wherein the vector is selected from an adeno-associated virus vector, an adenovirus vector, an RNA virus vector, a lentivirus vector, and a vaccinia virus vector.

8. A recombinant AAV vector having packaged therein an expression cassette comprising an engineered human complement regulator factor H (fH) gene operably linked to expression control sequences which direct expression thereof, wherein said hfH gene encodes a soluble hfH protein variant that retains complement regulatory function, wherein said fH variant comprises:

short consensus repeat (SCR) 1, 2, 3, 4, 19 and 20, wherein following administration of the vector to a subject and expression, detectable therapeutically useful plasma levels of the hfH variant are present in the subject for at least about a month.

9. The recombinant vector according to claim 8, wherein the detectable plasma levels of the hfH variant are present for at least about 6 months.

10. The recombinant vector according to claim 8, wherein detectable plasma levels are present for at least about 10 months.

11. The recombinant AAV vector according to claim 8, wherein the hfH variant further comprises additional hfH SCR consisting of (a) SCR7, SCR17 and/or SCR18 and (b) at least one of SCR6, SCR8, and SCR16, or combinations thereof.

12. The recombinant AAV vector according to claim 11, wherein the hfH variant comprises SCR domains SCR1-4, 7, 19, and 20.

13. The recombinant AAV vector according to claim 12, wherein the hfH variant comprises at least a linker of 1 to about 18 amino acids located between one or more of the SCRs.

14. The recombinant AAV vector according to claim 13, wherein the hfH variant comprises SCR1-(L1)- SCR2-(L2)-SCR3-(L3)-SCR4-(L4)-(SCR6-(L4'))-SCR7-(L5)-(SCR8-(L5'))-(SCR16-(L5"))-(SCR17-(L5"'))-(SCR18-(L5""))-SCR19-(L6)-SCR20, wherein the () indicate optional component, "L" refers to a linker, and each of L1, L2, L3, L4, L4', L5, L5', L5", L5"', L5"" and L6 may be absent or independently selected from an amino acid sequence of about 1 to about 12 to about 18 amino acids.

15. The recombinant AAV vector according to any one of claims 8 to 14, wherein the fH variant comprises at least one glycosylation site in one or more of the SCRs.

16. The recombinant AAV vector according to claim 8 to 15, wherein the recombinant AAV is designed to target the liver cells and has a capsid selected from an AAV8 capsid, rh64R1 capsid, AAV9 capsid, or rh10 capsid.

17. The recombinant AAV vector according to claim 16, wherein the expression cassette comprises a promoter which specifically directs expression of the hfH variant in liver cells.

18. The recombinant AAV vector according to any one of claims 8 to 15, wherein the recombinant vector is designed for expression in the eye, and the AAV capsid is selected from AAV1, AAV2, and AAV5.

19. The recombinant AAV vector according to claim 10, wherein the vector further comprises promoters tissue specific for the eye.

20. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a recombinant vector according to any one of claims 1-15, 18, and 19.

21. A method for treating a complement related disorder by delivering to the subject a vector according to any of claims 1 to 19.

22. The method according to claim 21, wherein the complement related disorder is C3 glomerulopathy including dense deposit disease and C3 glomerulonephritis, atypical hemolytic uremic syndrome (aHUS), age related macular degeneration (AMD), microangiopathic haemolytic anemia, thrombotic thrombocytopenia purpura (TTP), acute renal failure, paroxysmal nocturnal hemoglobinuria (PNH), schizophrenia, ischemic stroke, and/or bacterial infections caused by recruitment of bacterial pathogens.

23. The method according to claim 21, wherein said method comprises delivering the vector via a route selected from intravenous, intra-ocular, intramuscular, subcutaneous, or combinations thereof.

24. The method according to claim 21, wherein the method of treatment further comprises delivering protein-based fH therapy to said subject substantially simultaneously with the administration of the vector.

25. The method according to claim 21, wherein the complement mediated disorder is age-related macular degeneration.

26. The method according to claim 25, wherein the vector is administered subretinally.

27. The method according to claim 21, wherein the complement mediated disorder is a renal dysfunction.

28. A recombinant vector according to any one of claims 1 to 19 for use in a regimen for treating AMD.

29. A rAAV vector according to any one of claims 10 to 14 for use in a regimen for treating complement associated renal dysfunction.

30. An engineered hfH variant comprising human complement factor H SCRs consisting of:

(a) SCR1-4, 7, and 19-20;

(b) SCR1-4, 6, 7, and 19-20;

(c) SCR1-4, 7, 8, and 19-20;

(d) SCR1-4, 6, 7, 8, and 19-20;

(e) SCR1-4, 17, 19-20;

(f) SCR1-4, 18-20;

(g) SCR1-4, 17-20;

(h)SCR1-4, 7, and 18-20;

(i) SCR1-4, 6, 7, and 18-20;

(j) SCR1-4, 7, 8, and 18-20;

(k) SCR1-4, 6, 7, 8, and 18-20,

(l) SCR1-4, 7, and 17-20;

(m) SCR1-4, 6, 7, and 17-20;

(n) SCR1-4, 7, 8, and 17-20; or

(o) SCR1-4, 6, 7, 8, and 17-20.

31. The engineered hfH variant according to claim 30, wherein the hfH variant comprises SCR1-(L1)- SCR2-(L2)-SCR3-(L3)-SCR4-(L4)-(SCR6-(L4'))-SCR7-(L5)-(SCR8-(L5'))-(SCR16-(L5"))-(SCR17-(L5"'))-(SCR18-(L5""))-SCR19-(L6)-SCR20, wherein the () indicate optional component, "L" refers to a linker, and each of L1, L2, L3, L4, L4', L5, L5', L5", L5"', L5"" and L6 may be absent or independently selected from an amino acid sequence of about 1 to about 12-18 amino acids.

32. The engineered hfH variant according to any one of claim 30 (a) to (o), wherein at least one glycosylation site is engineered into at least one of the SCRs.

33. A pegylated hfH variant, wherein any one of the engineered hfH variants of claims 30 or claim 31 is pegylated.

34. A pharmaceutical composition comprising at least one type of the engineered hfH variant according to any of claims 30 to 34, a carrier and/or an excipient.

35. The pharmaceutical composition according to claim 34, wherein the carrier is a liposome.

36. The pharmaceutical composition according to claim 34, wherein the carrier is a nanocarrier.

37. The pharmaceutical composition according to claim 34, wherein the engineered hfH is pegylated.

Fig 1A


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Fig 1B

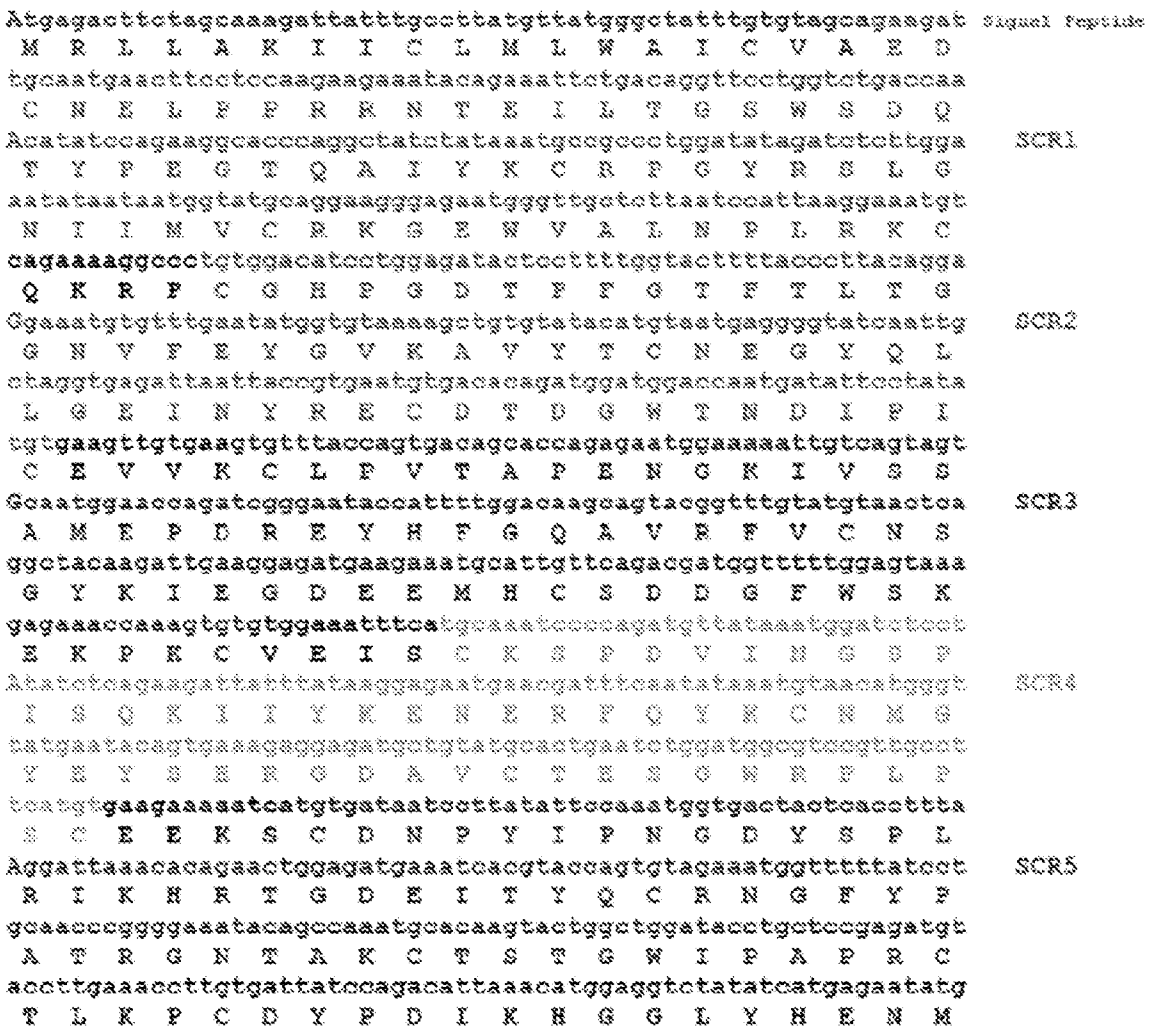
Signal Peptide
SCR1
SCR2
SCR3
SCR4
SCR5

FIG 1C

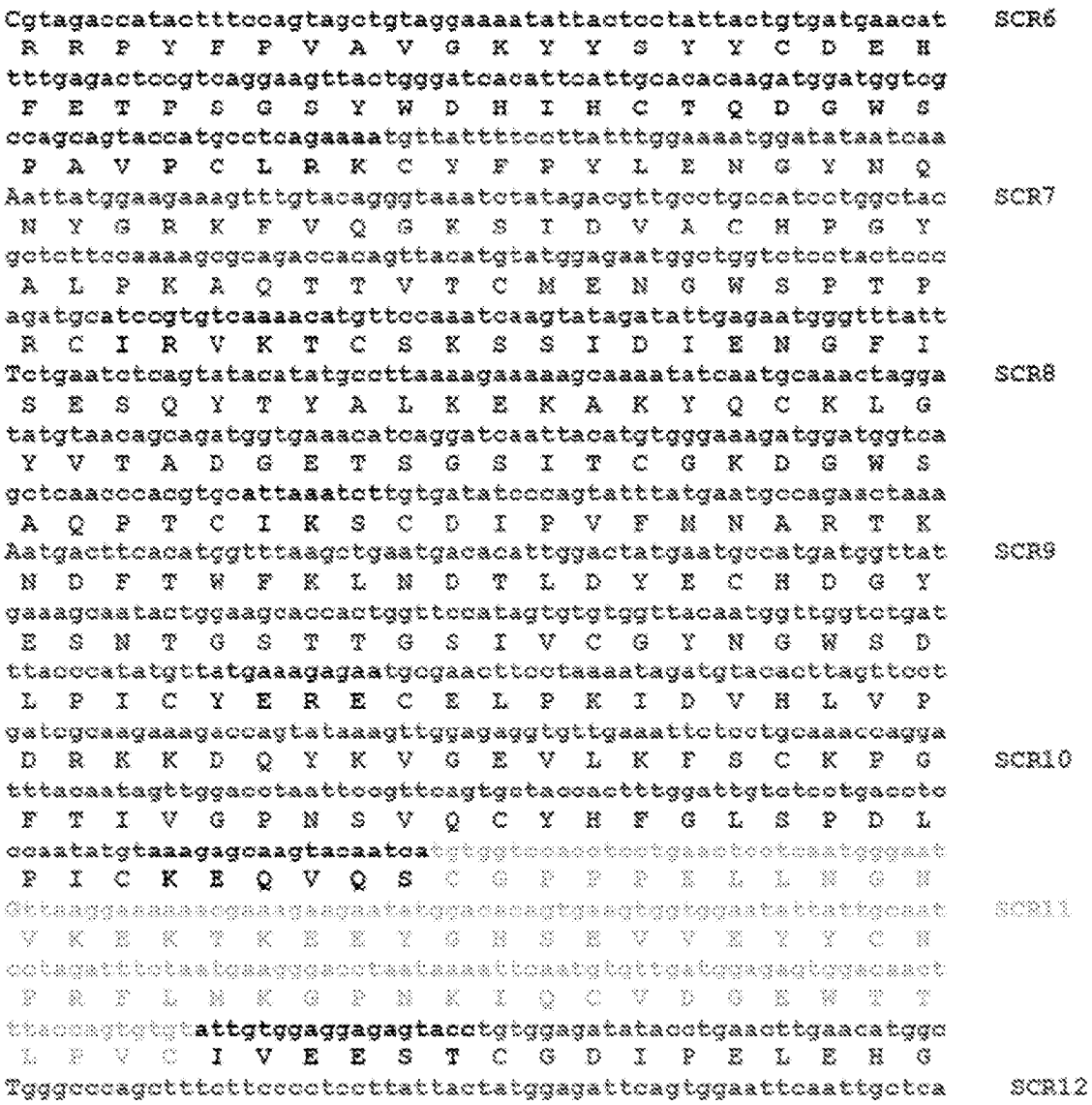

FIG 1D

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Cttgaggaacatttaaaaaacaagaaggaattcgatcataattctaacataaggtacaga      SCR13
 L  E  E  H  L  K  N  K  K  E  F  D  H  N  S  N  I  R  Y  R
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 C  R  G  K  E  G  W  I  H  T  V  C  I  N  G  K  W  D  P  E
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 V  N  C  S  M  A  Q  I  Q  L  C  P  P  P  P  Q  I  P  N  S
Cacaatatgacaaccacactgaattatcgggatggagaaaaagtatctgttctttgccaa      SCR14
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Fig 1E

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Acttcattcccgttgtcagtatatgctccagcttcatcagttgagtaccaatgccagaac      SCR19
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Fig 2A

FIG 2B

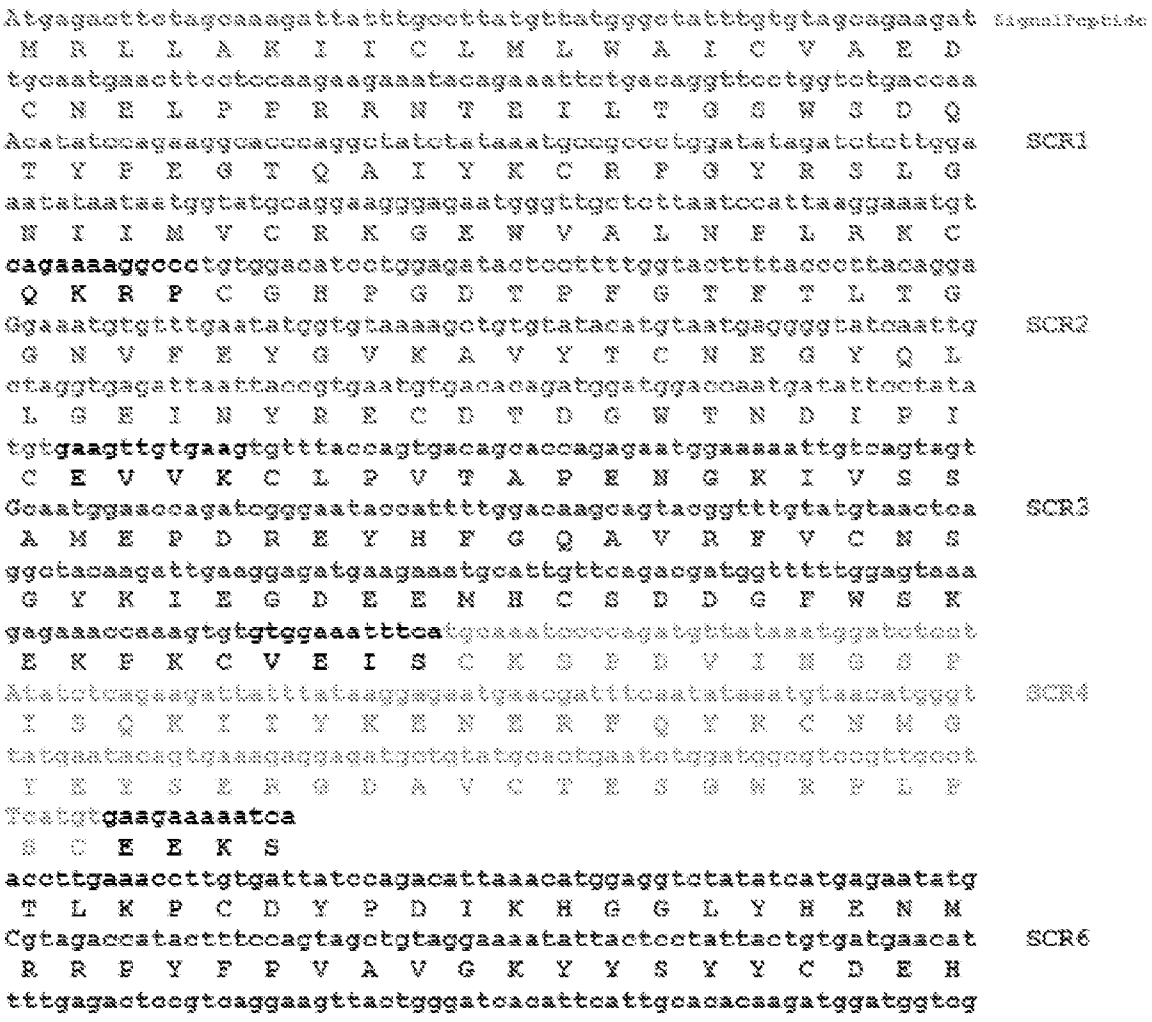

Fig 3A


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1102
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1242

Fig 3B

Aagtatagatattgagaatgggtttatttctgaatctcagtatacatatgccttaaaagaaaaagcgaaa
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1592

Aatgcttacatccgtgtgtaatatcccgagaaattatggaaaattataacatagcattaaggtggacagc
1662

Caaacagaagctttattcgagaacaggtgaatcagttgaatttgtgtgtaaacgggatatcgtctttca
1732

Tcacgttctcacacattgcgaacaacatgttgggatgggaaactggagtatccaacttgtgcaaaaagat
1802

agAATCAATCATAAAGTGCACACCTTTATTCAGAACTTTAGTATTAAATCAGTTCTCAATTTCATTTTTT
1872

ATGTATTGTTTTACTCCTTTTTATTCATACGTAAAATTTTGCATTAATTTGTGAAAATGTAATTATAAGC
1942

TGAGACCCGTGGCTCTCTTCTTAAAAGCACCATATTAAATCCTGCAAAACTAAAAAAAAAAAAAAAAAAA
2012

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 2068

FIG 3C

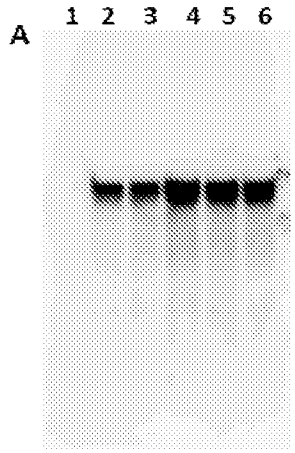
```
 F  E  T  P  S  G  S  Y  W  D  H  I  H  C  T  Q  D  G  W  S
ccagcagtaccatgcctcagaaaaatgttatttccttatttggaaaatggatataatcaa
 P  A  V  P  C  L  R  K  C  Y  F  P  Y  L  E  N  G  Y  N  Q
Aattatggaagaaagtttgtacagggtaaatctatagacgttgcctgccatcctggctac    SCR7
 N  Y  G  R  K  F  V  Q  G  K  S  I  D  V  A  C  H  P  G  Y
gctcttccaaaagcgcaagaccacagttacatgtatggagaatggctggtctcctactccc
 A  L  P  K  A  Q  T  T  V  T  C  M  E  N  G  W  S  P  T  P
agatgcatccgtgtcaaaacatgttccaaatcaagtatagatattgagaatgggtttatt
 R  C  I  R  V  K  T  C  S  K  S  S  I  D  I  E  N  G  F  I
Tctgaatctcagtatacatatgccttaaaagaaaaagcaaaaatatcaatgccaaactagga   SCR8
 S  E  S  Q  Y  T  Y  A  L  K  E  K  A  K  Y  Q  C  K  L  G
tatgtaacagcagatggtgaaacatcaggatcaattacatgtgggaaagatggatggtcca
 Y  V  T  A  D  G  E  T  S  G  S  I  T  C  G  K  D  G  W  S
gctcaacccacgtgcattaaatct
 A  Q  P  T  C  I  K  S
aaagattctacaggaaaatgtgggccccctccacctattgacaatggggacatt
 K  D  S  T  G  K  C  G  P  P  P  P  I  D  N  G  D  I
Acttcattcccgttgtcagtatatgctccagcttcatcagttgagtaccaatgccagaac    SCR19
 T  S  F  P  L  S  V  Y  A  P  A  S  S  V  E  Y  Q  C  Q  N
ttgtatcaacttgagggtaacaagcgaataacatgtagaaatggacaatggtcagaacca
 L  Y  Q  L  E  G  N  K  R  I  T  C  R  N  G  Q  W  S  E  P
ccaaaatgcttacatccgtgtgtaatatcccgagaaattatggaaaattataacatagca
 P  K  C  L  H  P  C  V  I  S  R  E  I  M  E  N  Y  N  I  A
Ttaaggtggacagccaaacagaagctttattcgagaacaggtgaatcagttgaatttgtg    SCR20
 L  R  W  T  A  K  Q  K  L  Y  S  R  T  G  E  S  V  E  F  V
tgtaaacggggatatcgtctttcatcacgttctcacacattgcgaacaaacatgttgggat
 C  K  R  G  Y  R  L  S  S  R  S  H  T  L  R  T  T  C  W  D
gggaaaatggagtatccaacttgtgcaaaaagatag
 G  K  L  E  Y  P  T  C  A  K  R  -
```

Fig 4

hfH1-4.678.19-20 protein amino acid sequence

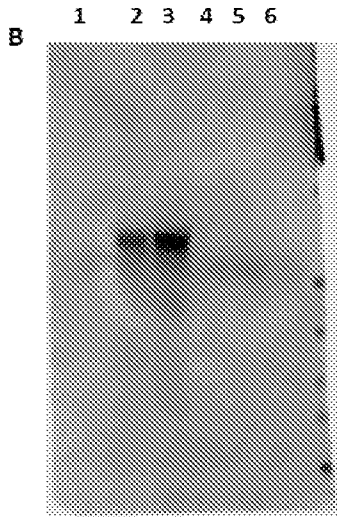
MRLLAKIICLMLWAICVAEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPGYRSLGNVIMVCRKGE
WVALNPLRKCQKRPCGHPGDTPFGTFTLTGGNVFEYGVKAVYTCNEGYQLLGEINYRECDTDGWTNDI
PICEVVKCLPVTAPENGKIVSSAMEPDREYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVE
ISCKSPDVINGSPISQKIIYKENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSTLKPCDYPDIKHG
GLYHENMRRPYFPVAVGKYYSYYCDEHFETPSGSYWDHIHCTQDGWSPAVPCLRKCYFPYLENGYNQN
HGRKFVQGKSIDVACHPGYALPKAQTTVTCMENGWSPTPRCIRVKTCSKSSIDIENGFISESQYTYALKEK
AKYQCKLGYVTADGETSGSIRCGKDGWSAQPTCIKSKDSTGKCGPPPPIDNGDITSFPLSVYAPASSVEYQ
CQNLYQLEGNKRITCRNGQWSEPPKCLHPCVISREIMENYNIALRWTAKQKLYSRTGESVEFVCKRGYRL
SSRSHTLRTTCWDGKLEYPTCAKR

Fig 5A



1:Con
2:pCMV Sport6- 1
3:pCMV Sport6-2
4:pCBARBG-1
5:pCBARBG-2
6:pCBARBG-3

Fig 5B



1:Con
2:pCBARBG-1
3:pAAV Cl1
4:pAAV Cl2
5:pAAV Cl3
6:pAAV Cl4

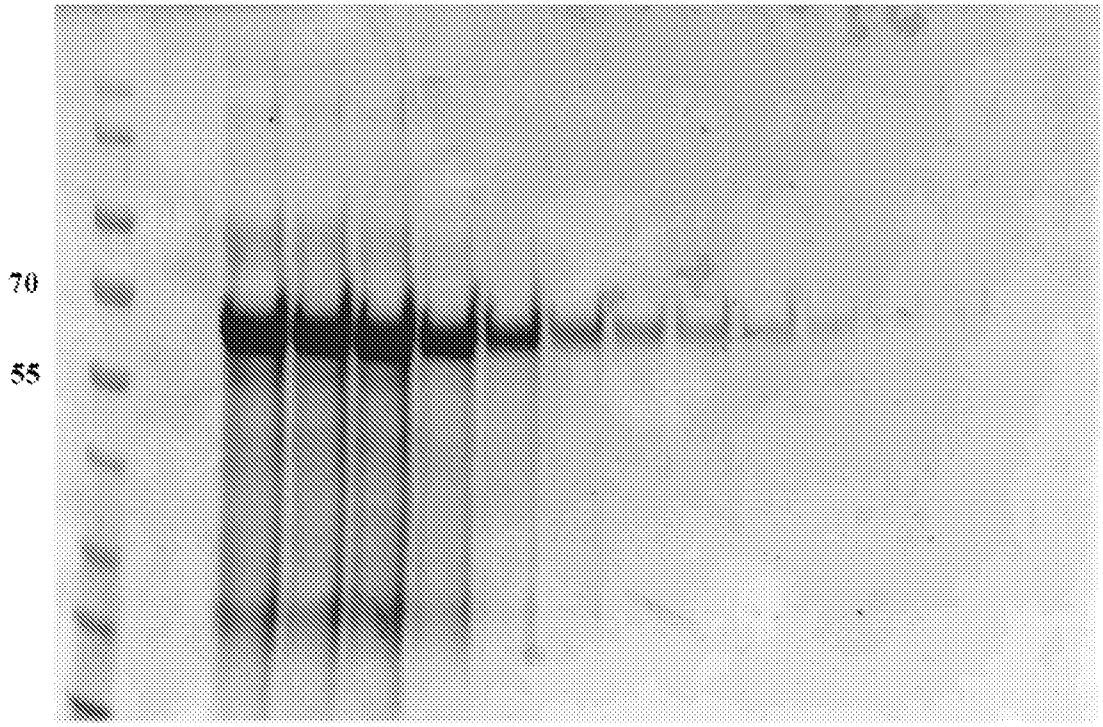

70
55

Fig 6

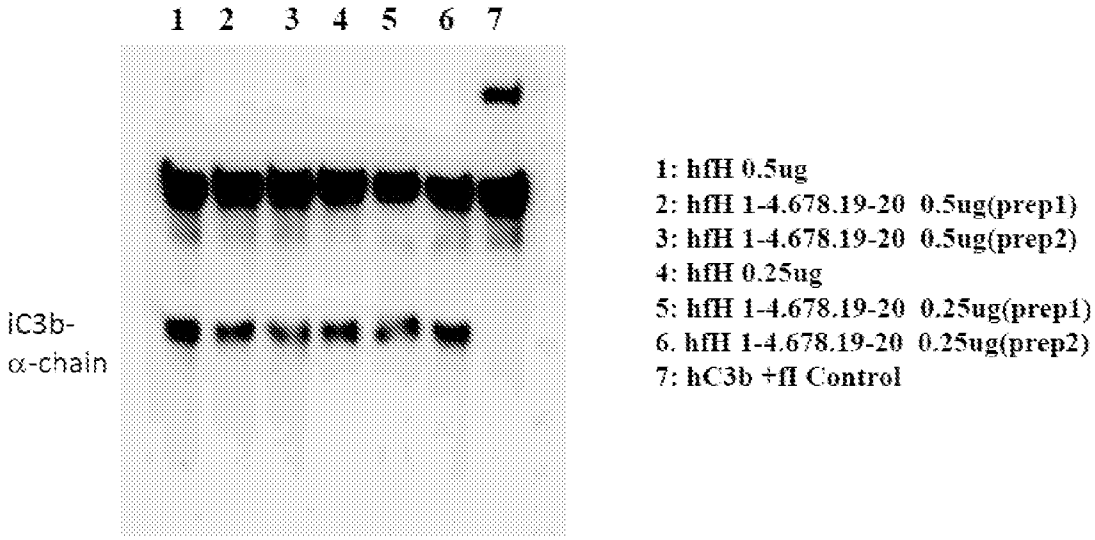

1 2 3 4 5 6 7
iC3b-
α-chain
1: hfH 0.5ug
2: hfH 1-4.678.19-20 0.5ug(prep1)
3: hfH 1-4.678.19-20 0.5ug(prep2)
4: hfH 0.25ug
5: hfH 1-4.678.19-20 0.25ug(prep1)
6. hfH 1-4.678.19-20 0.25ug(prep2)
7: hC3b +fI Control

Fig 7

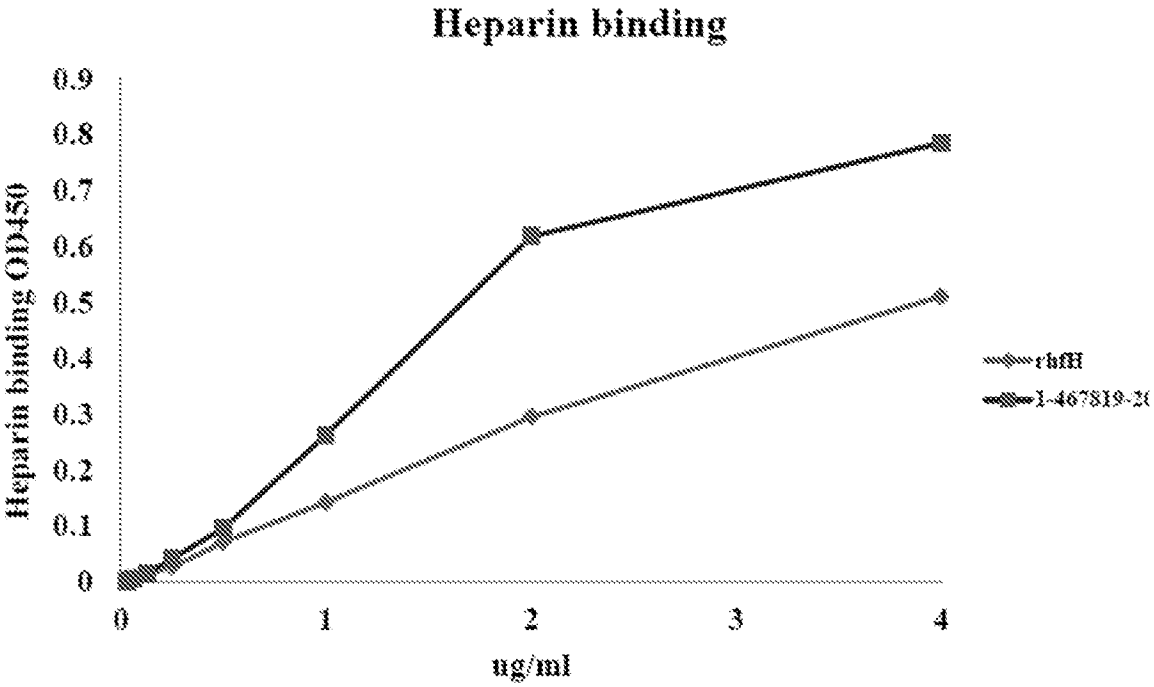

Heparin binding
Heparin binding OD450
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
ug/ml
rhfH
1-467819-2(

Fig 8

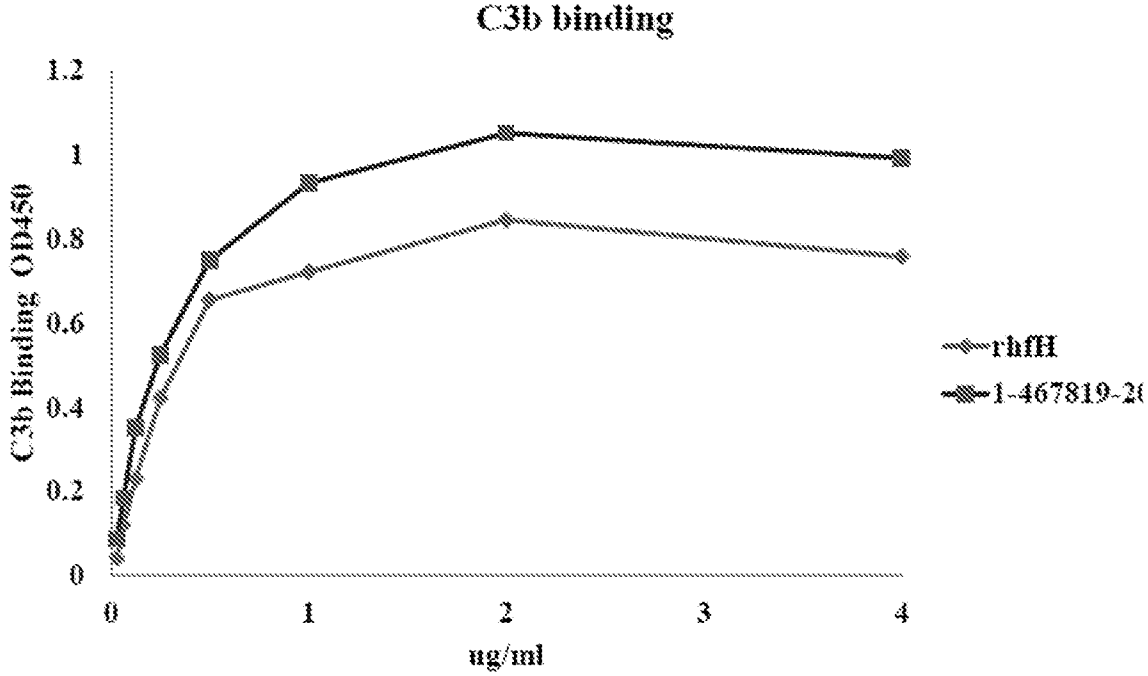

C3b binding
C3b Binding OD450
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
ug/ml
rhfH
1-467819-2(

Fig 9

FIG 10

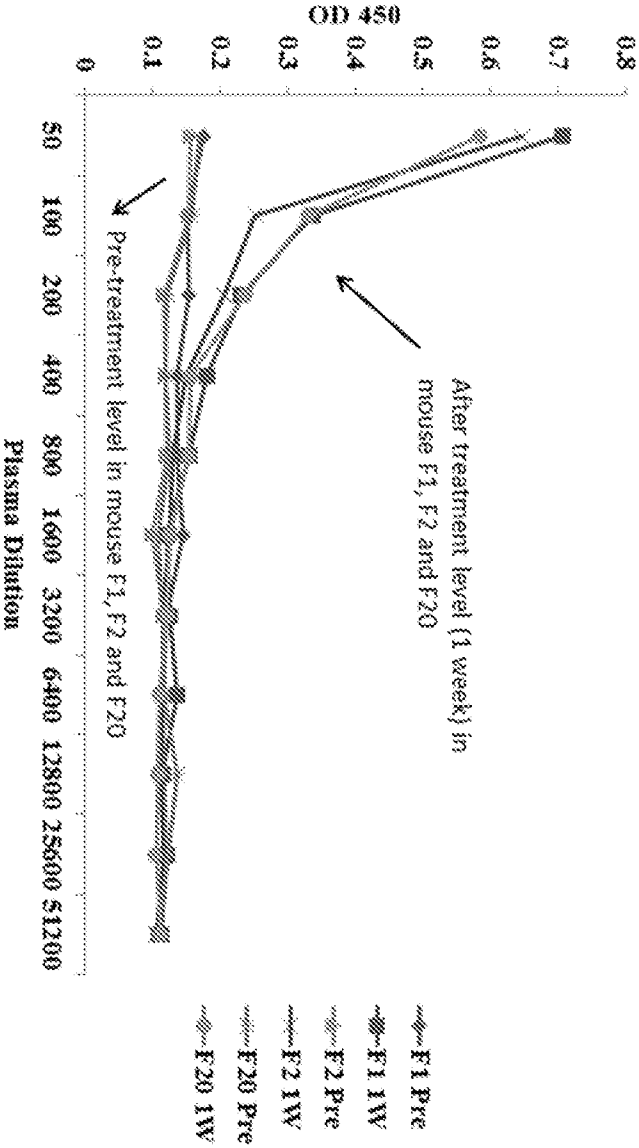

OD 450
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
50 100 200 400 800 1600 3200 6400 12800 25600 51200
Plasma Dilution
Pre-treatment level in mouse F1, F2 and F20
After treatment level (1 week) in mouse F1, F2 and F20
F1 Pre
F1 1W
F2 Pre
F2 1W
F20 Pre
F20 1W

FIG 11

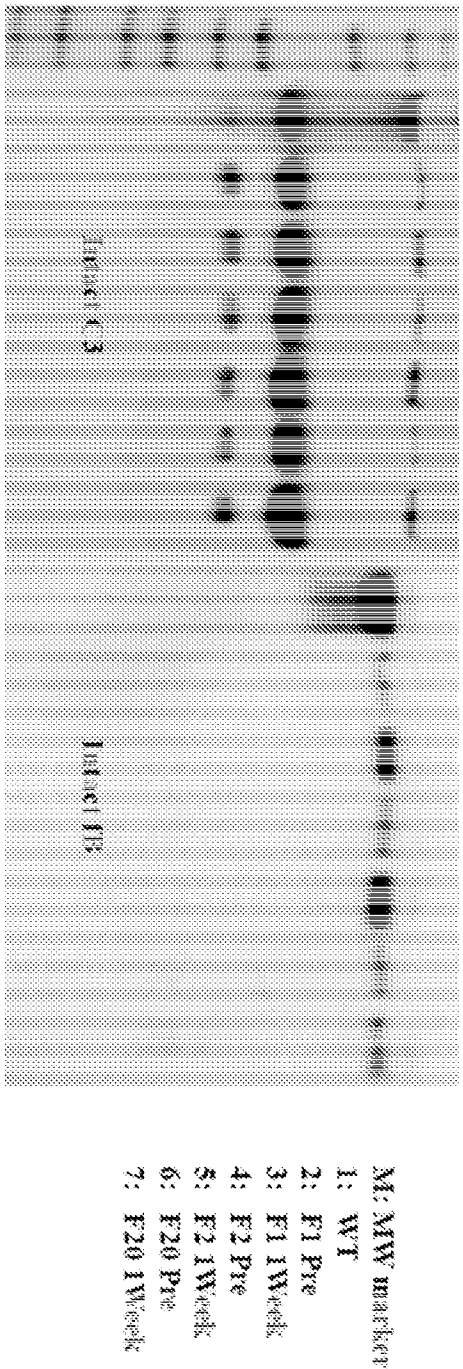

M 1 2 3 4 5 6 7 1 2 3 4 5 6 7
Intact IC3
Intact IB
M: MW marker
1: WT
2: F1 Pre
3: F1 1Week
4: F2 Pre
5: F2 1Week
6: F20 Pre
7: F20 1Week

FIG 12A

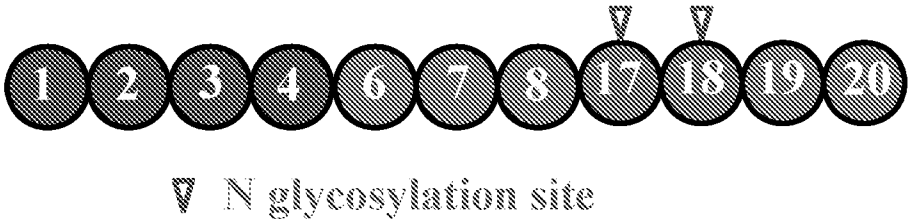


FIG 12B

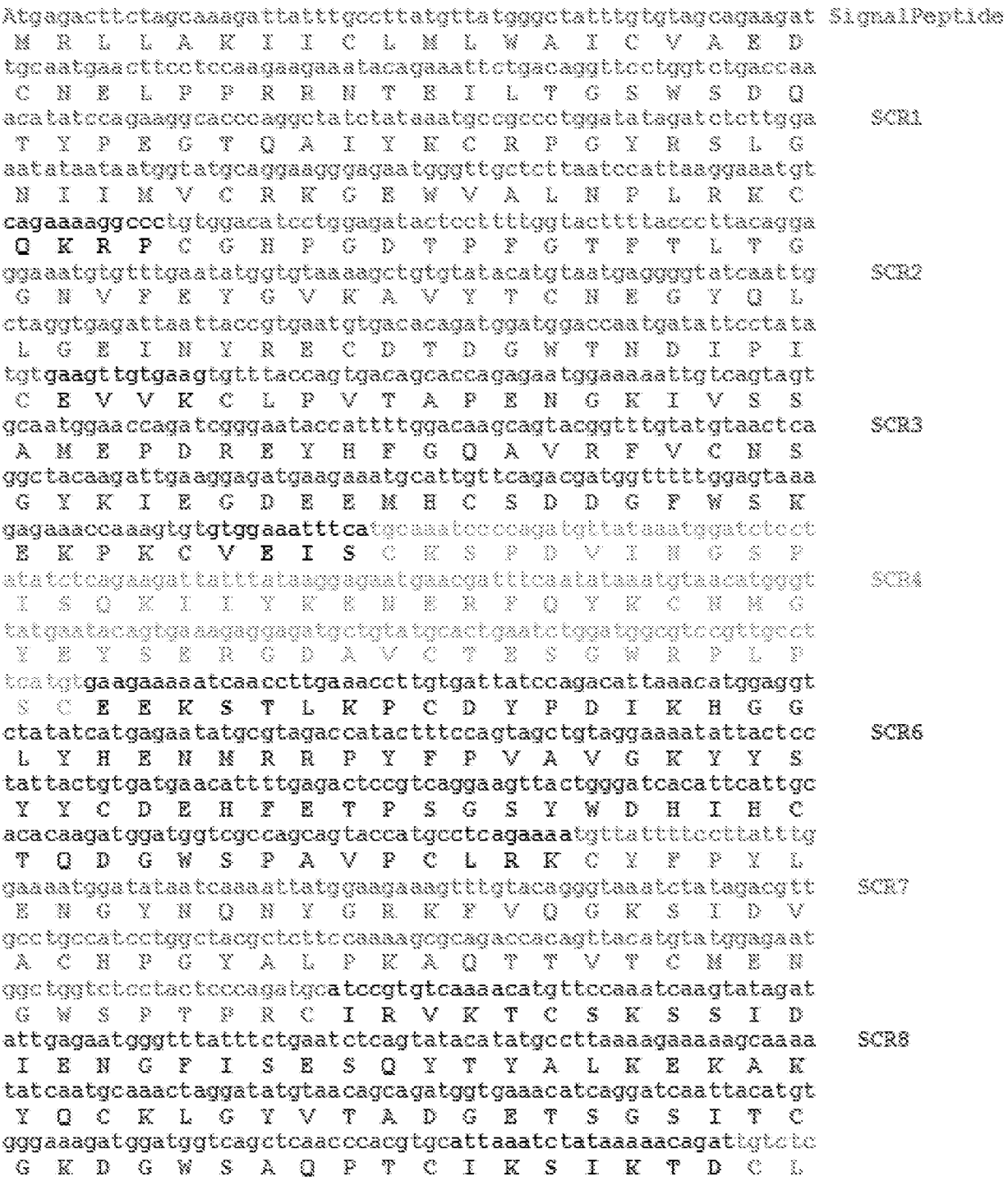

Atgagacttctagcaaagattatttgccttatgttatgggctatttgtgtagcagaagat SignalPeptide
M R L L A K I I C L M L W A I C V A E D
tgcaatgaacttcctccaagaagaaatacagaaattctgacaggttcctggtctgaccaa
C N E L P P R R N T E I L T G S W S D Q
acatatccagaaggcacccaggctatctataaatgccgccctggatatagatctcttgga SCR1
T Y P E G T Q A I Y K C R P G Y R S L G
aatataataatggtatgcaggaagggagaatgggttgctcttaatccattaaggaaatgt
N I I M V C R K G E W V A L N P L R K C
cagaaaaggccctgtggacatcctggagatactcctttggtactttaccccttacagga
Q K R P C G H P G D T P F G T F T L T G
ggaaatgtgtttgaatatggtgtaaaagctgtgtatacatgtaatgaggggtatcaattg SCR2
G N V F E Y G V K A V Y T C N E G Y Q L
ctaggtgagattaattaccgtgaatgtgacacagatggatggaccaatgatattcctata
L G E I N Y R E C D T D G W T N D I P I
tgtgaagttgtgaagtgtttaccagtgacagcaccagagaatggaaaaattgtcagtagt
C E V V K C L P V T A P E N G K I V S S
gcaatggaaccagatcgggaataccattttggacaagcagtacggtttgtatgtaactca SCR3
A M E P D R E Y H F G Q A V R F V C N S
ggctacaagattgaaggagatgaagaaatgcattgttcagacgatggtttttggagtaaa
G Y K I E G D E E M H C S D D G F W S K
gagaaaccaaagtgtgtggaaatttcatgcaaatcccagatgttataaatggatctcct
E K P K C V E I S C K S P D V I N G S P
atatctcagaagattatttataaggagaatgaacgatttcaatataaatgtaacatgggt SCR4
I S Q K I I Y K E N E R F Q Y K C N M G
tatgaatacagtgaaagaggagatgctgtatgcactgaatctggatggcgtccagttgcct
Y E Y S E R G D A V C T E S G W R P L P
tcatgtgaagaaaaatcaaccttgaaacctgtgattatccagacattaaacatggaggt
S C E E K S T L K P C D Y P D I K H G G
ctatatcatgagaatatgcgtagaccatactttccagtagctgtaggaaaatattactcc SCR6
L Y H E N M R R P Y F P V A V G K Y Y S
tattactgtgatgaacattttgagactccgtcaggaagttactgggatcacattcattgc
Y Y C D E H F E T P S G S Y W D H I H C
acacaagatggatggtcgccagcagtaccatgcctcagaaaatgttatttttccttatttg
T Q D G W S P A V P C L R K C Y F P Y L
gaaaatggatataatcaaaattatggaagaaagtttgtacagggtaaatctatagacgtt SCR7
E N G Y N Q N Y G R K F V Q G K S I D V
gcctgccatcctggctacgctcttccaaaagcgcagaccacagttacatgtatggagaat
A C H P G Y A L P K A Q T T V T C M E N
ggctggtctcctactcccagatgcatccgtgtcaaaacatgttccaaatcaagtatagat
G W S P T P R C I R V K T C S K S S I D
attgagaatgggtttatttctgaatctcagtatacatatgccttaaaagaaaaagcaaaa SCR8
I E N G F I S E S Q Y T Y A L K E K A K
tatcaatgcaaactaggatatgtaacagcagatggtgaaacatcaggatcaattacatgt
Y Q C K L G Y V T A D G E T S G S I T C
gggaaagatggatggtcagctcaacccacgtgcattaaatctataaaaacagattgtctc
G K D G W S A Q P T C I K S I K T D C L

Fig 12C

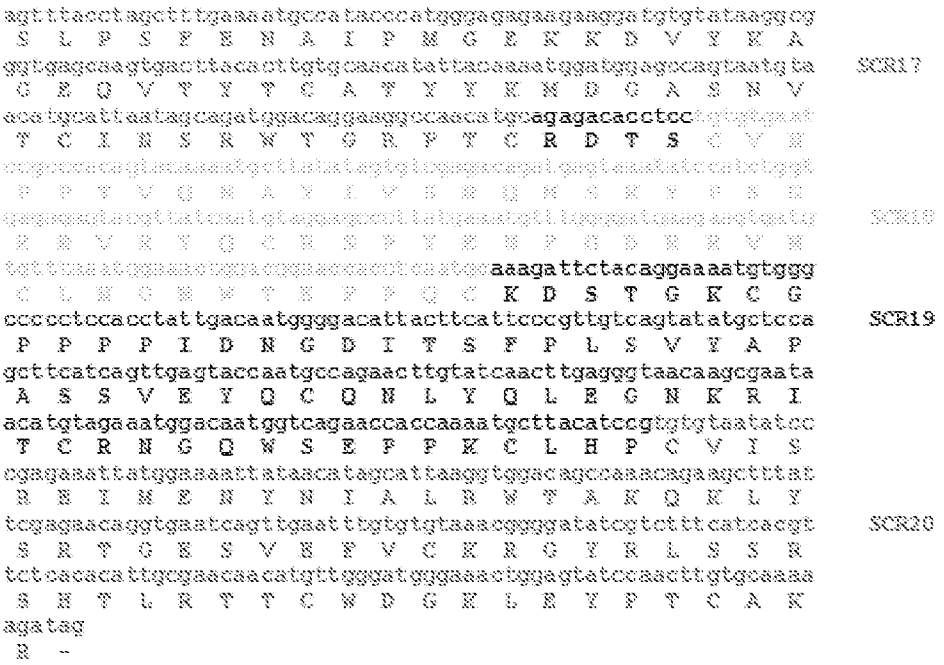


Fig 13

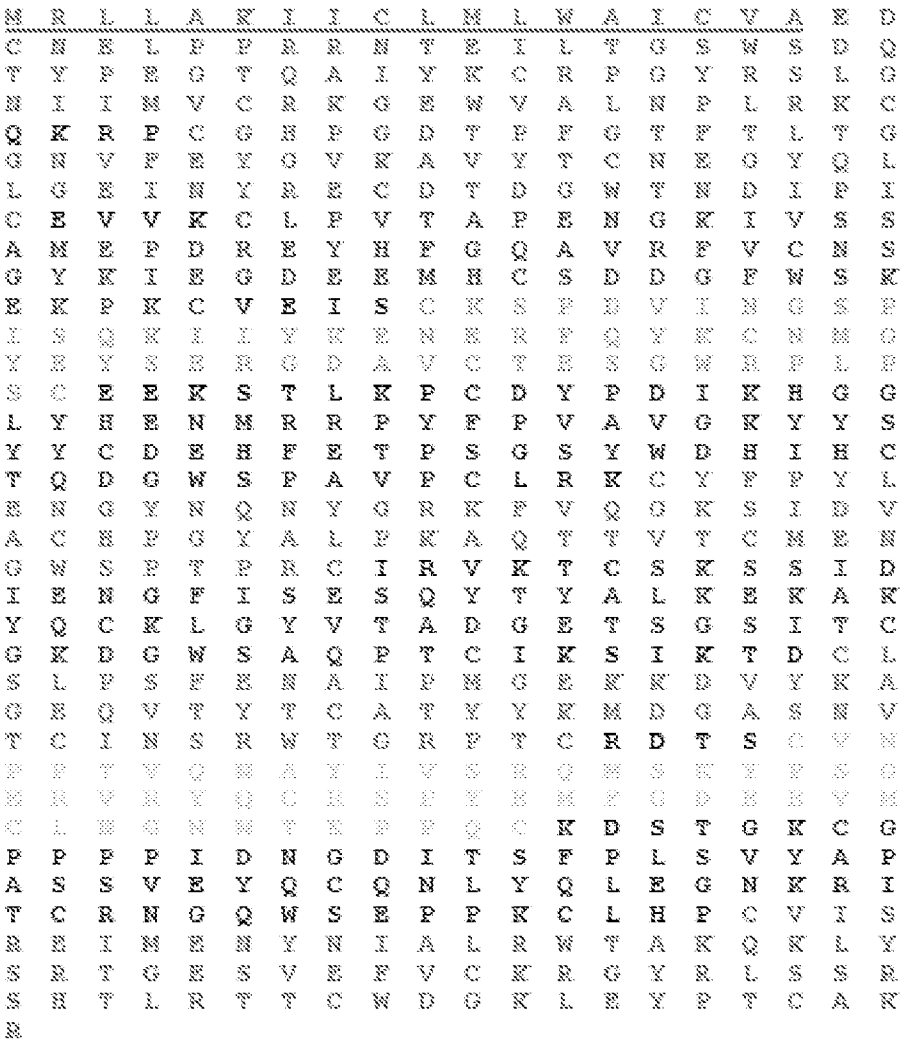


Fig 14

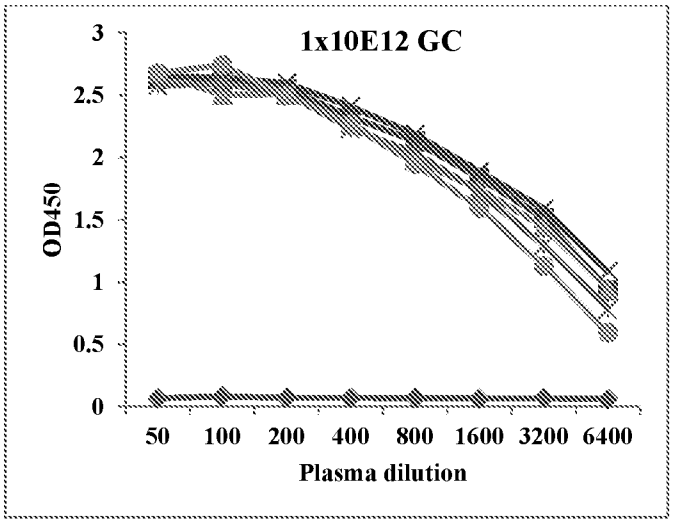


Fig 15A

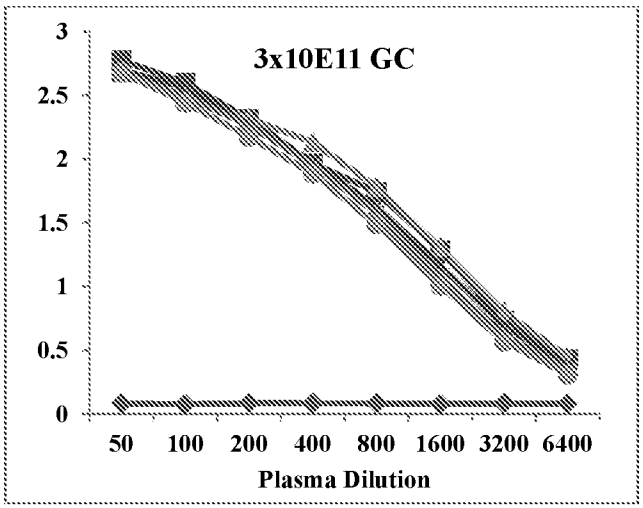


Fig 15B

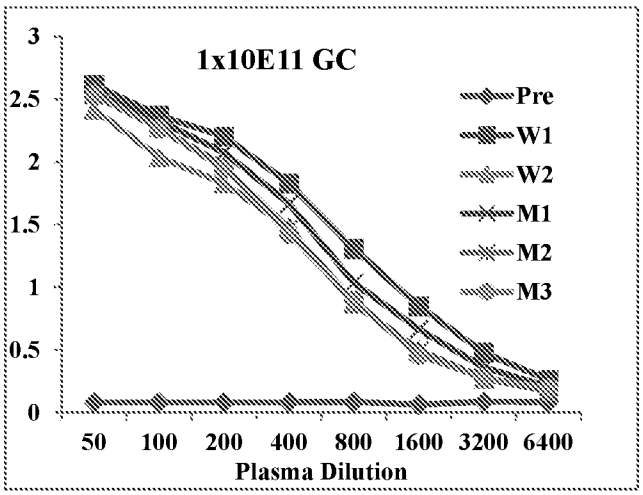


Fig 15C

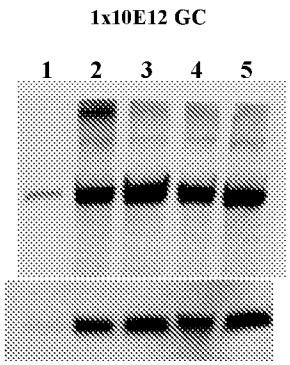
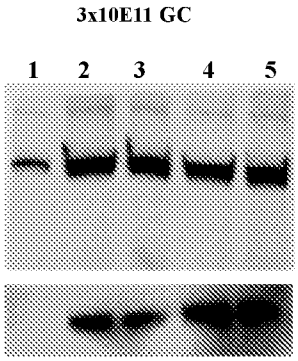


FIG. 16A



1: Pre
2: 1 Week post AAV
3: 1 Month post AAV
4: 2 Months post AAV
5: 3 Months post AAV

FIG I6B

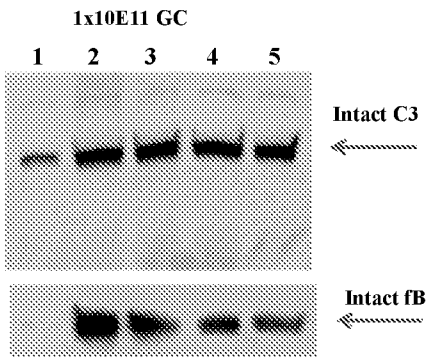


FIG 16C

FIG 17A

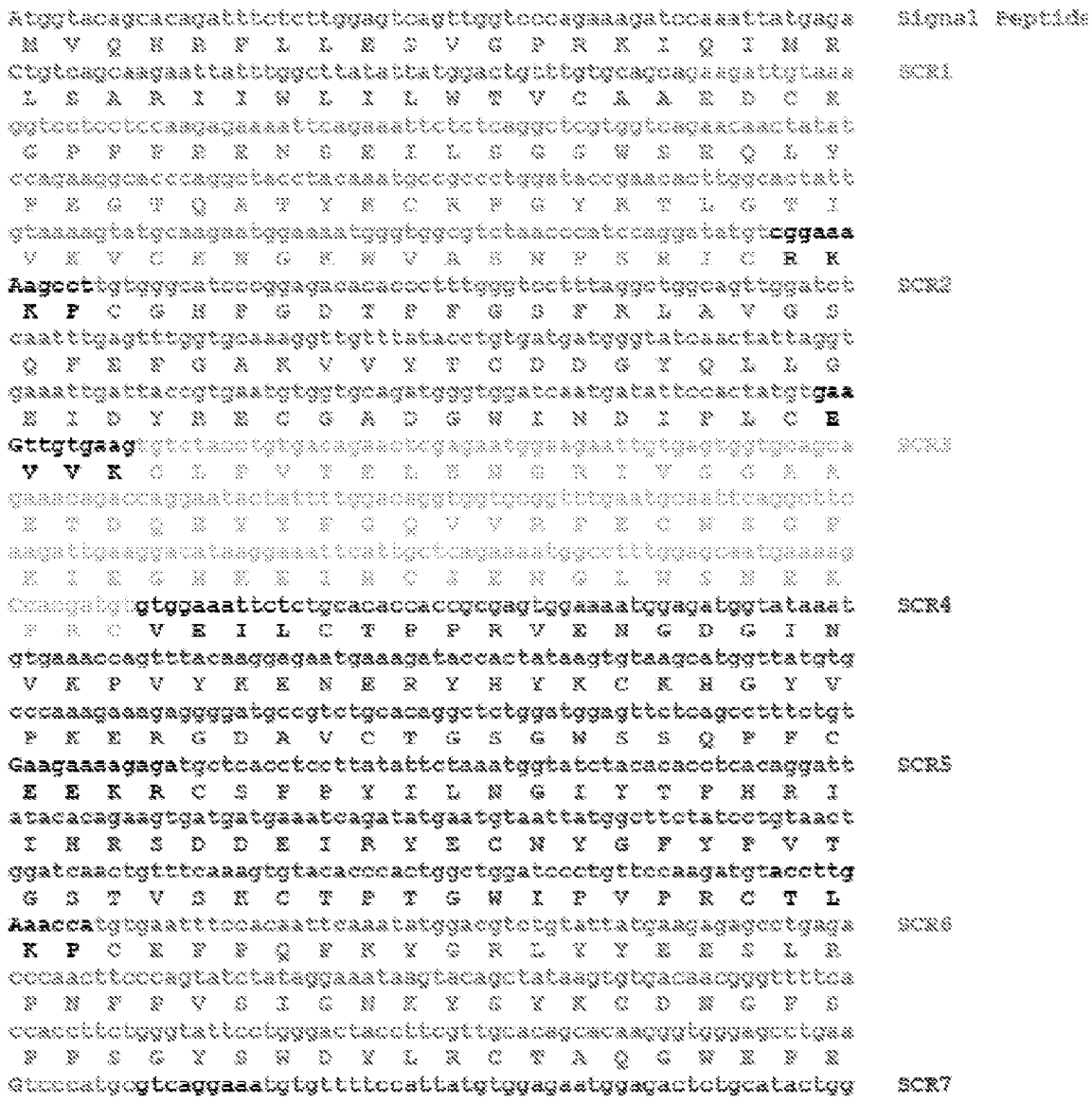
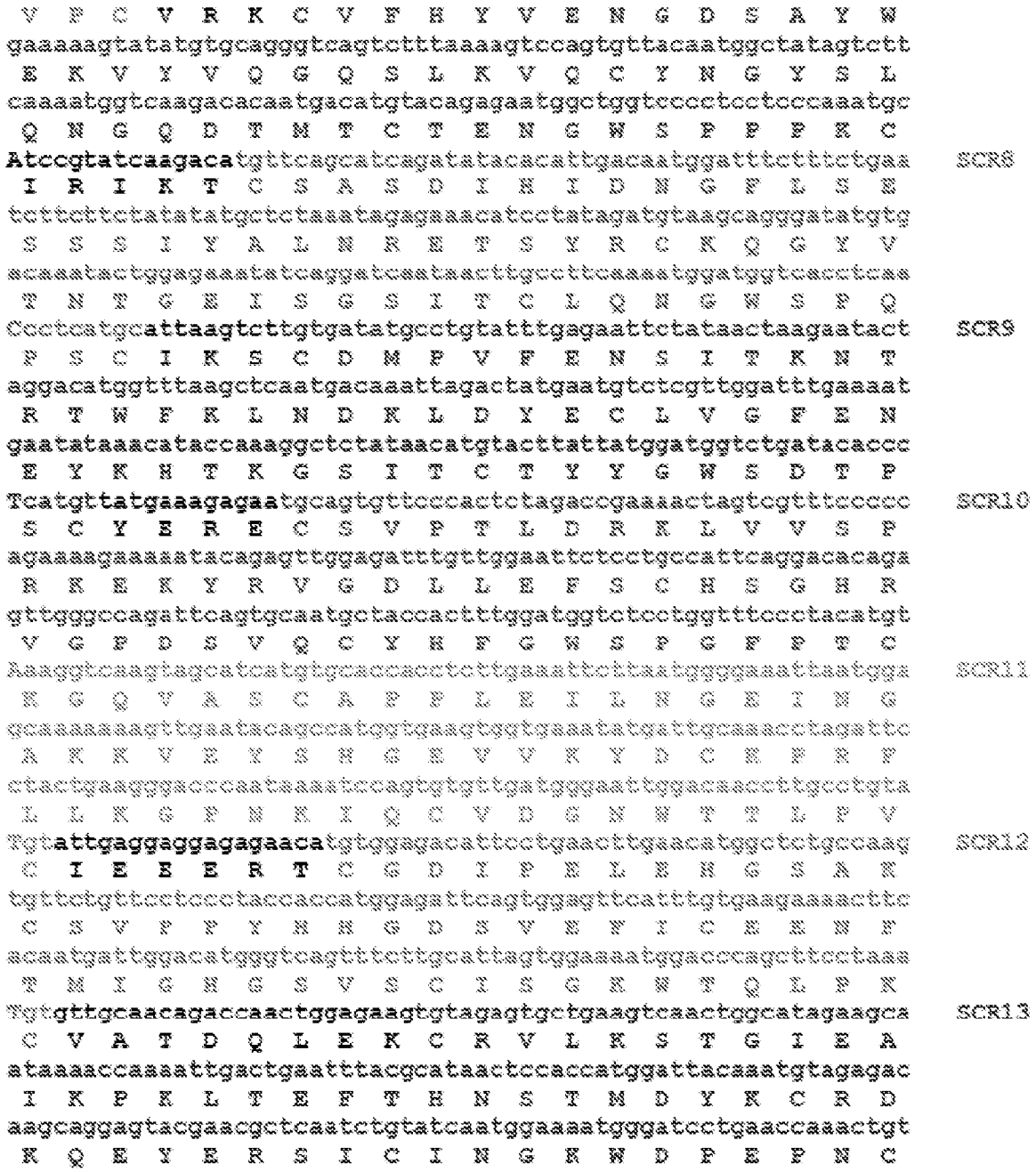


Fig 17B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E K V Y V Q G Q S L K V Q C Y N G Y S L
caaaatggtcaagacacaatgacatgtacagagaatggctggtcccctcctcccaaatgc
Q N G Q D T M T C T E N G W S P P P K C
Atccgtatcaagaca tgttcagcatcagatatacacattgacaatggatttctttctgaa SCR8
I R I K T C S A S D I N I D N G F L S E
tcttcttctatatatgctctaaatagagaaacatcctatagatgtaagcagggatatgtg
S S S I Y A L N R E T S Y R C K Q G Y V
acaaatactggagaaatatcaggatcaataacttgccttcaaaatggatggtcaccctcaa
T N T G E I S G S I T C L Q N G W S P Q
Ccctcatgc attaagtcttgtgatatgcctgtatttgagaattctataactaagaatact SCR9
P S C I K S C D M P V F E N S I T K N T
aggacatggtttaagctcaatgacaaattagactatgaatgtctcgttggatttgaaaat
R T W F K L N D K L D Y E C L V G F E N
gaatataaacataccaaaggctctataacatgtacttattatggatggtctgatacaccc
E Y K H T K G S I T C T Y Y G W S D T P
Tcatgttatgaaagagaa tgcagtgttcccactctagaccgaaaactagtcgtttccccc SCR10
S C Y E R E C S V P T L D R K L V V S P
agaaaagaaaaatacagagttggagatttgttggaattctcctgccattcaggacacaga
R K E K Y R V G D L L E F S C H S G H R
gttgggccagattcagtgcaatgctaccactttggatggtctcctggtttccctacatgt
V G P D S V Q C Y H F G W S P G F P T C
Aaaggtcaagtagcatcatgtgcaccacctcttgaaattcttaatgggggaaattaatgga SCR11
K G Q V A S C A P P L E I L N G E I M G
gcaaaaaaagttgaatacagccatggtgaagtggtgaaatatgattgcaaacctagattc
A K K V E Y S H G E V V K Y D C K P R F
ctactgaagggacccaataaaatccagtgtgttgatgggaattggacaaccttgcctgta
L L K G P N K I Q C V D G N W T T L P V
Tgtattgaggaggagagaaca tgtggagacattcctgaacttgaacatggctctgccaag SCR12
C I E E E R T C G D I P E L E H G S A K
tgttctgttcctcccctaccaccatggagattcagtggagttcatttgtgaagaaaacttc
C S V P P Y H H G D S V E F I C E E N F
acaatgattggacatgggtcagtttcttgcattagtggaaaatggacccagcttcctaaa
T M I G H G S V S C I S G K W T Q L P K
Tgtgttgcaacagaccaactggagaagtgtagagtgctgaagtcaactggcatagaagca SCR13
C V A T D Q L E K C R V L K S T G I E A
ataaaaccaaaattgactgaatttacgcataactccaccatggattacaaatgtagagac
I K P K L T E F T H N S T M D Y K C R D
aagcaggagtacgaacgctcaatctgtatcaatggaaaatgggatcctgaaccaaactgt
K Q E Y E R S I C I N G K W D P E P N C

Fig 17C

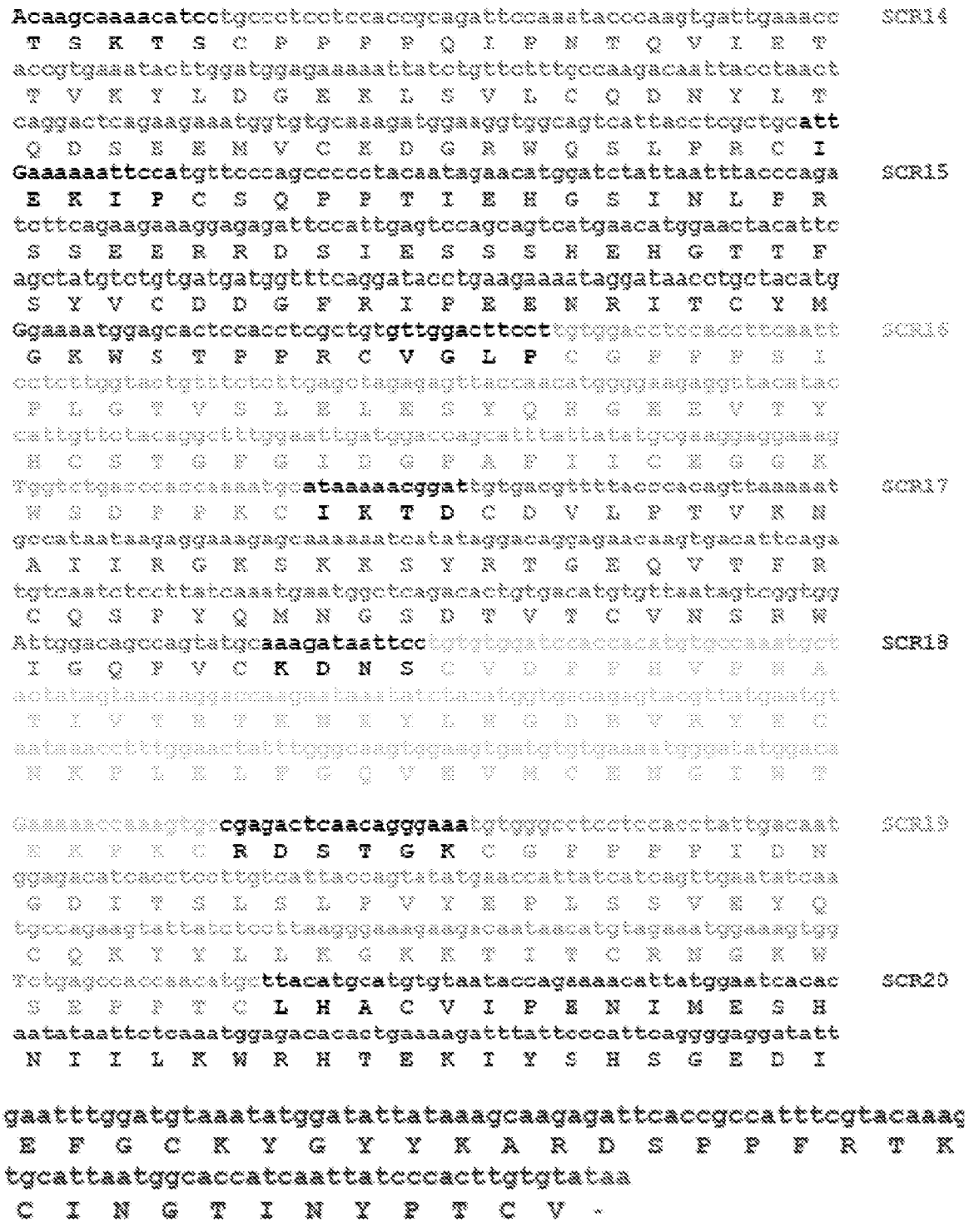

Fig 18A

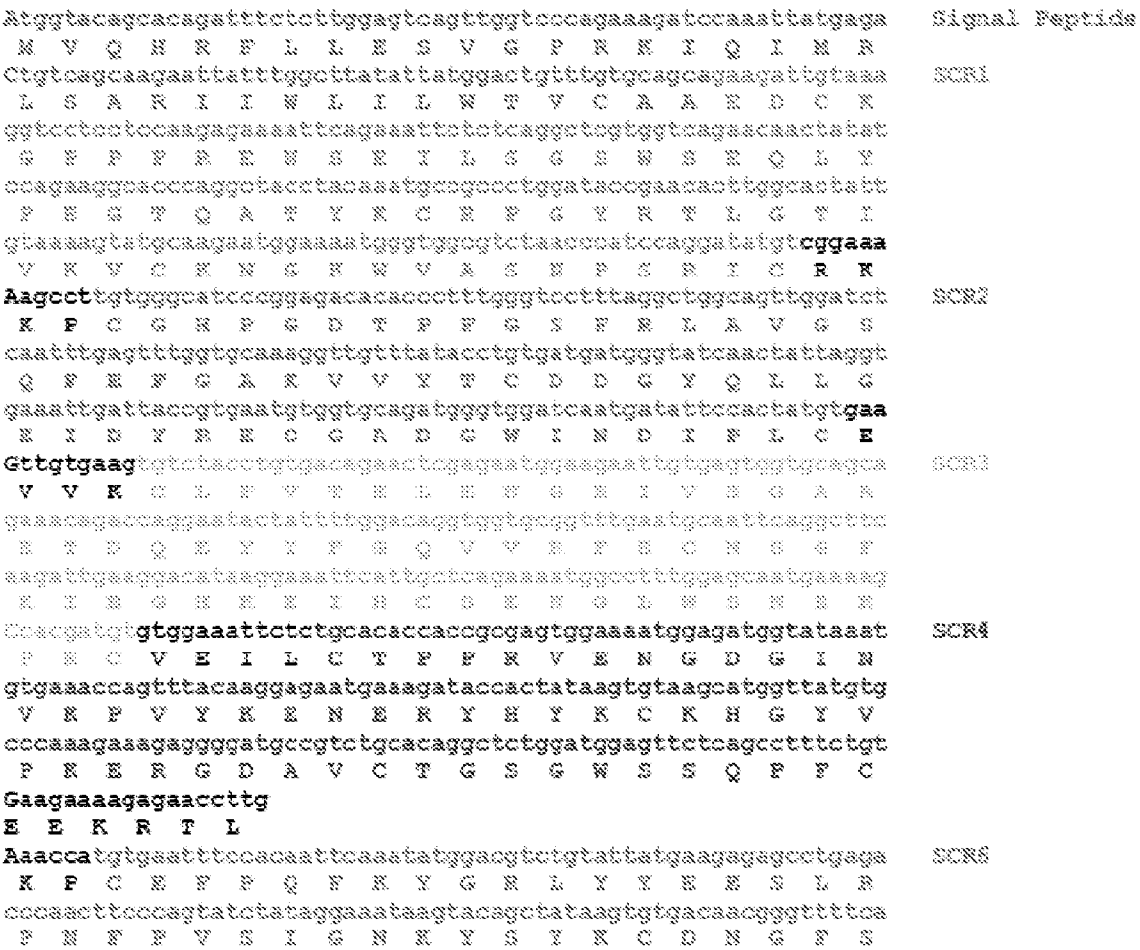

Fig 18B

```
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Gtcccatgcgtcaggaaatgtgttttccattatgtggagaatggagactctgcatactgg    SCR7
 V  P  C  V  R  K  C  V  F  H  Y  V  E  N  G  D  S  A  Y  W
gaaaaagtatatgtgcagggtcagtctttaaaagtccagtgttacaatggctatagtctt
 E  K  V  Y  V  Q  G  Q  S  L  K  V  Q  C  Y  N  G  Y  S  L
caaaatggtcaagacacaatgacatgtacagagaatggctggtcccctcctcccaaatgc
 Q  N  G  Q  D  T  M  T  C  T  E  N  G  W  S  P  P  P  K  C
Atccgtatcaagacatgttcagcatcagatatacacattgacaatggatttctttctgaa    SCR8
 I  R  I  K  T  C  S  A  S  D  I  H  I  D  N  G  F  L  S  E
tctttcttctatatatgctctaaatagagaaacatcctatagatgtaagcagggatatgtg
 S  S  S  I  Y  A  L  N  R  E  T  S  Y  R  C  K  Q  G  Y  V
acaaatactggagaaatatcaggatcaataacttgccttcaaaatggatggtcacctcaa
 T  N  T  G  E  I  S  G  S  I  T  C  L  Q  N  G  W  S  P  Q
Ccctcatgcattaagtct
 P  S  C  I  K  S
cgagactcaacagggaaatgtgggcctcctccacctattgacaat                   SCR19
 R  D  S  T  G  K  C  G  P  P  P  P  I  D  N
ggagcatcacctccttgtcattaccagtatatgaaccattatcatcagttgaatatcaa
 G  D  I  T  S  L  S  L  P  V  Y  E  P  L  S  S  V  E  Y  Q
tgccagaagtattatctccttaagggaaagaagacaataacatgtagaaatggaaagtgg
 C  Q  K  Y  Y  L  L  K  G  K  R  T  I  T  C  R  N  G  K  W
Tctggccaccaacatgcttacatgcatgtgtaataccagaaaacattatggaatcacac    SCR20
 S  G  P  P  T  C  L  H  A  C  V  I  P  E  N  I  M  E  S  H
aatataattctcaaatggagacacactgaaaagatttattcccattcaggggaggatatt
 N  I  I  L  K  W  R  H  T  E  K  I  Y  S  H  S  G  E  D  I
gaatttggatgtaaatatggatattataaagcaagagattcaccgccatttcgtacaaag
 E  F  G  C  K  Y  G  Y  Y  K  A  R  D  S  P  P  F  R  T  K
tgcattaatggcaccatcaattatcccacttgtgtataa
 C  I  N  G  T  I  N  Y  P  T  C  V  -
```

Fig 19

GGTCTACTATTTAGTTTACTTTGCAGAAGTTGCTCATGGGCGGAGCAATCCTGATTTCCTAAACTGACTTCAACTTCCCTTTGAAGCAAGTCTTT
CCCTGCTGTGACCACAGTTCATAGCAGAGAGGAACTGGATGGTACAGCACAGATTTCTCTTTGGAGTCAGTTGGTCCCAGAAAGATCCAAA
TTATGAGACTGTCAGCAAGAATTATTTGCTTATATTATGACTGTTTGTGCAGCAGAAGATTGTAAAGGTCCTCCTCCAAGAGAAAA
TTCAGAAATTCTCTCAGCTCGTCATCAGACAACTATATCCAGAAGGCACCCAGCTACCTACAAATGCCGCCCTGGATACCGAACA
CTTGGCACTATTGTAAAAGTATGCAAGAATGGAAAATGGGTGGCTCTAACCCATCCAGGATATGTCGGAAAAAGCCTTGTGGGCATC
CCGGAGACACACCCTTTGGTCCTTTAGGCTGGCAGTTGGATCTCAATTTGAGTTTGTTGGTGCAAAGGTTGTTATTACCTGTGATGATGG
GTATCAACTATTAGGTGAAATTGATTACCGTGAATGTGGTGCAGATGGGTGGATCAATGATATTCCACTATGTGAAGTTGTGAAGTGT
CTACCTGTGACAGAACTCGAGAATGGAAGAATTGTGAGTGGTGCAGCAGAAACAGACCAGGAATACTATTTGAACAGGTGGTGCGGT
TTGAATGCAATTCAGGCTTCAAGATTGAAGGACATAAGGAAATTCATTGCTCAGAAAATGGCCTTTGGAGCAATGAAAAGCCACGATG
TGTGGAAATTCTCTGCACACCACCGCGAGTGGAAAATGGATGGTATAAATGTGAAACCAGTTTACAAGGAGAATGAAAGATACCAC
TATAAGTGTAAGCATGGTTATGTGCCCAAAGAAAGAGGGATGCCGTCTGCACAGGCTCTGGATGGAGTTCTCAGCCTTTCTGTGAAG
AAAAGAGAACCTTGAACCATGTGAATTTCCACAATTCAAATATGGACGTCTGTATTATGAAGAGACCCTGAGACCCAACTTCCCAGT
ATCTATAGGAAATAAGTACAGCTATAAGTGTGACAACGGTTTTCACCACCTTCTGGGACTACCTTCGTTGTTGCACAGCA
CAAGGGTGGTAGCCTGAAGTCCCATGCGTCAGGAATGTGTTTCCATATGTCTTCAAAATGGTCAAGACACAATGACTACTGGAAAAAGTAT
ATGTGCAGGGTCAGTCTTTAAAAGTCCAGTGTTACAATGGCTATAGTCTTCAAAATGGTCAAGACACAATGACTACTGGAAAAAGTGG
CTGGTCCCCCTCCTCCCAAATGCATCCGTATCAAGACATGTTCAGCATCAGATATACACATTGACAATGGATTTCTTTCTTGAATCTTCT
TCTATATATGCTCTAAATAGAGAAACATCCTATAGATGTAAGCAGGGATATGTGACAAATACTGGAGAAATACAGGATCAATAACTT
GCCTTCAAAATGGATGGTCACCTCAACCCTCATGCATTAAGTCTCGAGACTCAACAGGGAAATGTGGCCCTCCTCCACCTATTGACAA
TGGAGACATCACCTCCTTGTCATTACCAGTATATGAACCATTATCATCAGTTGAATATCAATGCCAGAAGTATTATCCTTAAGGGA
AAGAAGACAATAACATGTAGAAATGGAAAGTGGTCTGAGCCACCAACATGCTTACATGCATGTGTAATACCAGAAAACATTATGGAAT
CACACAATATAATTCTCAAATGGAGACACTGAAAAAGATTTATTCCATTCAGGGGAGGATATTGGATTTGGATGTAAATATGGATA
TTATAAAGCAAGATTCACCGCCATTTCGTACAAAGTGCATTAATGGCACCATCAATTATCCCACTTGTGTACAAAATCATAATACA
TTTATTAGTTGATTTTATTGTTTAGAAAGGCACATGCATGTGACTAATATACTTTCAATTTGCATTGAAGTATTGTTTAACTCATGTC
TTCTCATAAAATATAAACATTTTTGTTATATGGTGATTAATTTGTAAAAACTATTGCCAAAATGCAAAAGCAGTAATTCAAAA
CTCCTAATCTAAAATATGATATGTCCAAGGACAAACTATTTCAATCAAGAAAGTAGATGTAAGTTCTTCAACATCTGTTTCTATTCAG
AACTTTCTCAGATTTCCTGGATACCTTTTGATGTAAGGTCCTGATTTACAGTGGATAAAGGATATATTGACTGATTCTCAAATTAA
TATGATTTCCCAAGCATGTAACAACCAACTATCATATATTATATGACTAATGCATACAATTAATTACTATATAATACTTTCAAATA
AAAGAATCTAAGAAACTTC

Fig 20

MVQHRFLLESVGPRKIQIMRLSARIIWLILWTVCAAEDCKGPPPREN
SEILSGSWSEQLYPEGTQATYKCRPGYRTLGTIVKVCKNGKWVASNP
SRICRKKPCGHPGDTPFGSFRLAVGSQFEFGAKVVYTCDDGYQLLGEI
DYRECGADGWINDIPLCEVVKCLPVTELENGRIVSGAAETDQEYYFG
QVVRFECNSGFKIEGHKEIHCSENGLWSNEKPRCVEILCTPPRVENG
DGINVKPVYKENERYHYKCKHGYVPKERGDAVCTGSGWSSQPFCEEK
RTLKPCEFPQFKYGRLYYEESLRPNFPVSIGNKYSYKCDNGFSPPSGYS
WDYLRCTAQGWEPEVPCVRKCVFHYVENGDSAYWEKVYVQGQSLK
VQCYNGYSLQNGQDTMTCTENGWSPPPKCIRIKTCSASDIHIDNGFL
SESSSIYALNRETSYRCKQGYVTNTGEISGSITCLQNGWSPQPSCIKSR
DSTGKCGPPPPIDNGDITSLSLPVYEPLSSVEYQCQKYYLLKGKKTITC
RNGKWSEPPTCLHACVIPENIMESHNIILKWRHTEKIYSHSGEDIEFG
CKYGYYKARDSPPFRTKCINGTINYPTCV

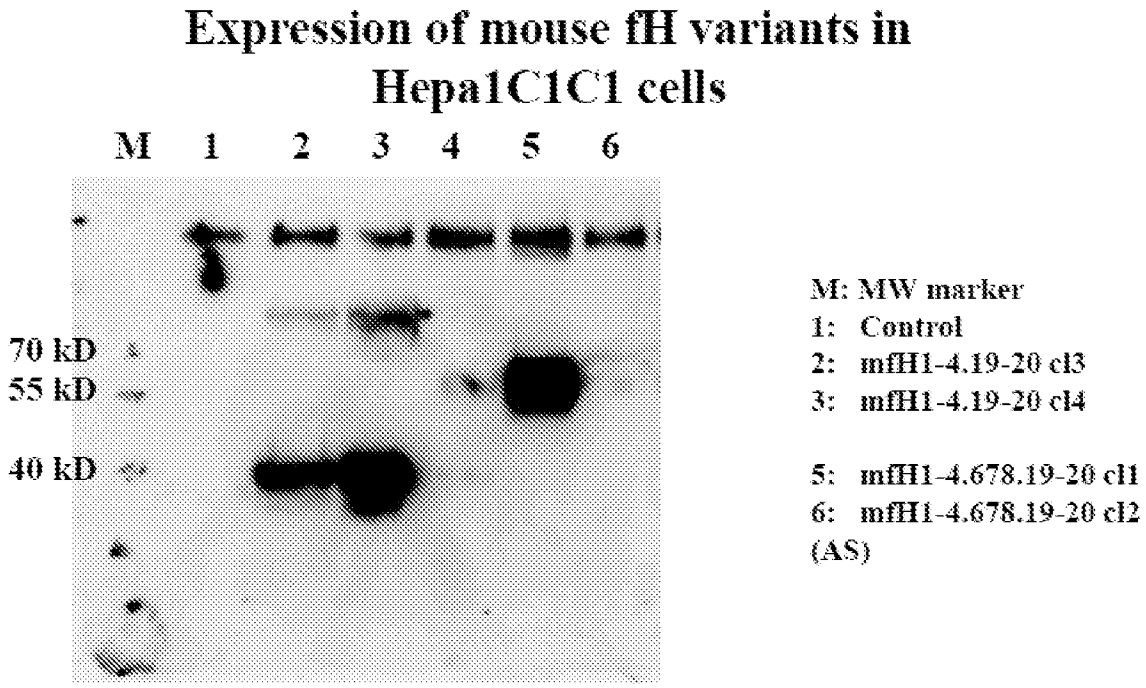


FIG. 21

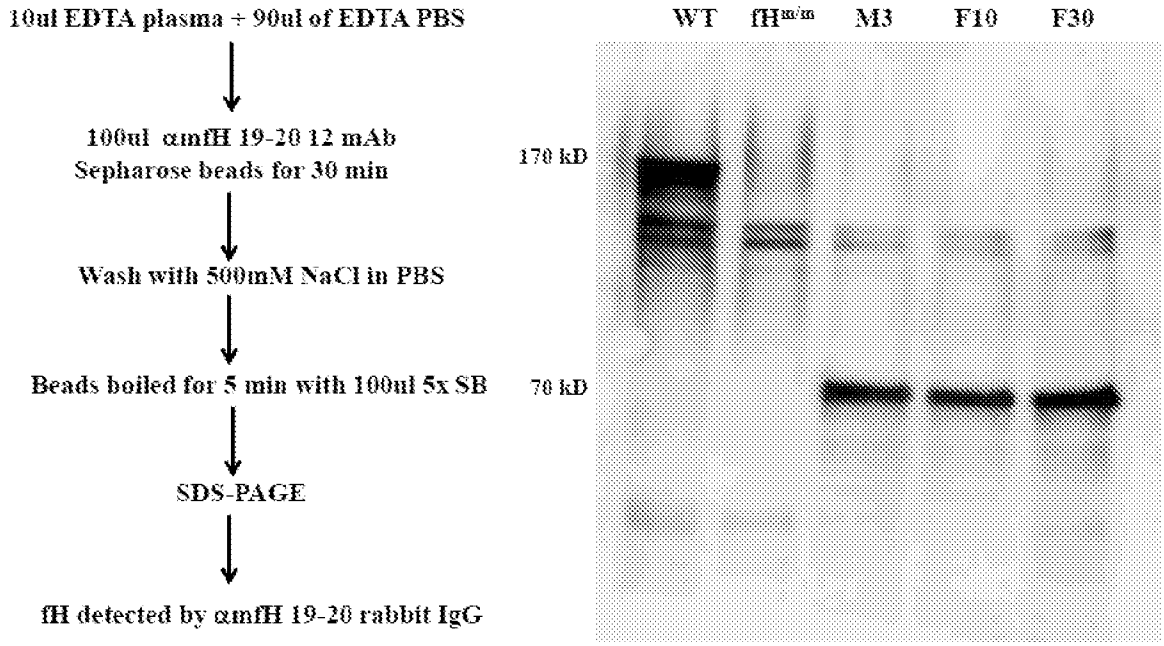


FIG 22A FIG 22B

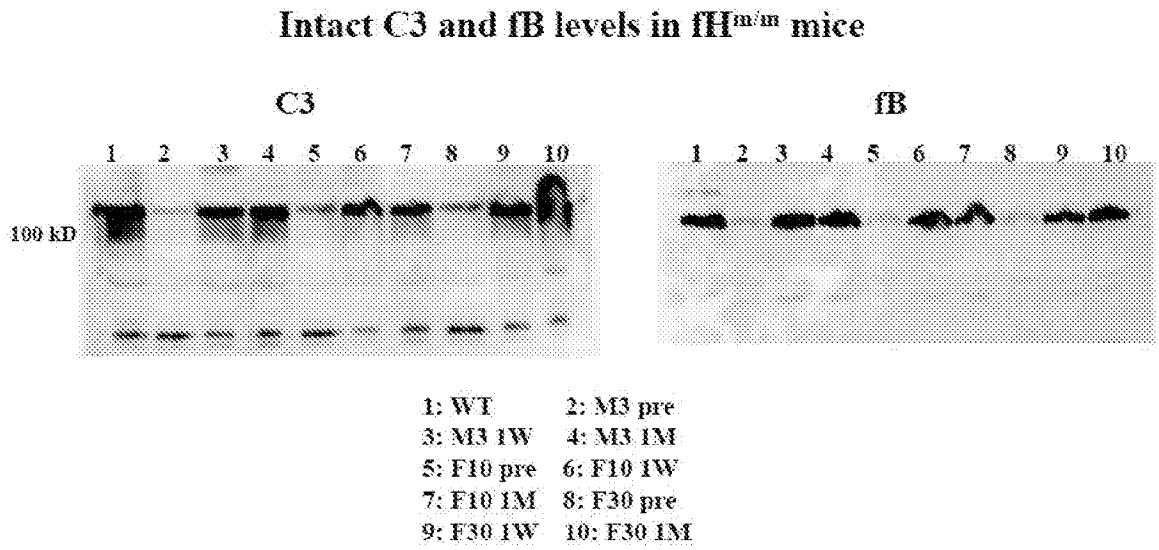

Intact C3 and fB levels in fH$^{m/m}$ mice
C3
fB
1 2 3 4 5 6 7 8 9 10
100 kD
1: WT 2: M3 pre
3: M3 1W 4: M3 1M
5: F10 pre 6: F10 1W
7: F10 1M 8: F30 pre
9: F30 1W 10: F30 1M

Fig 23

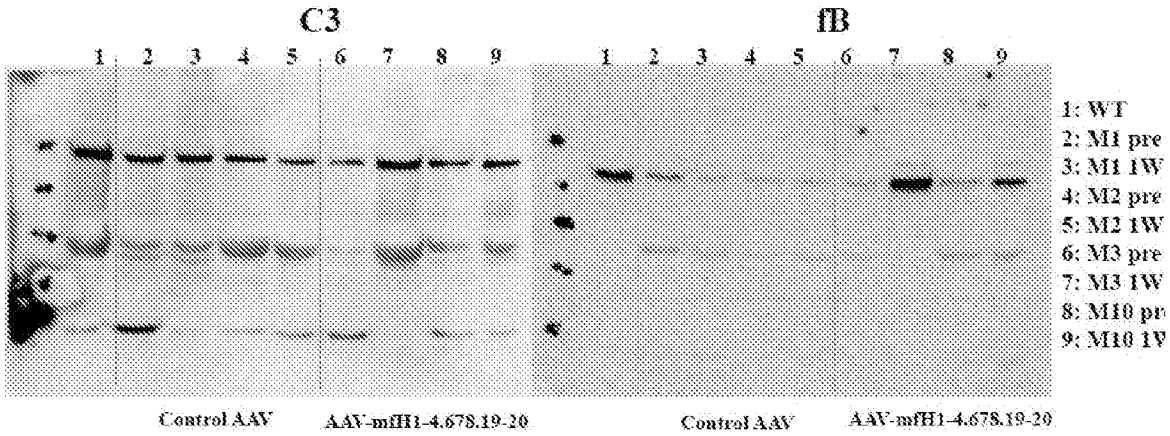
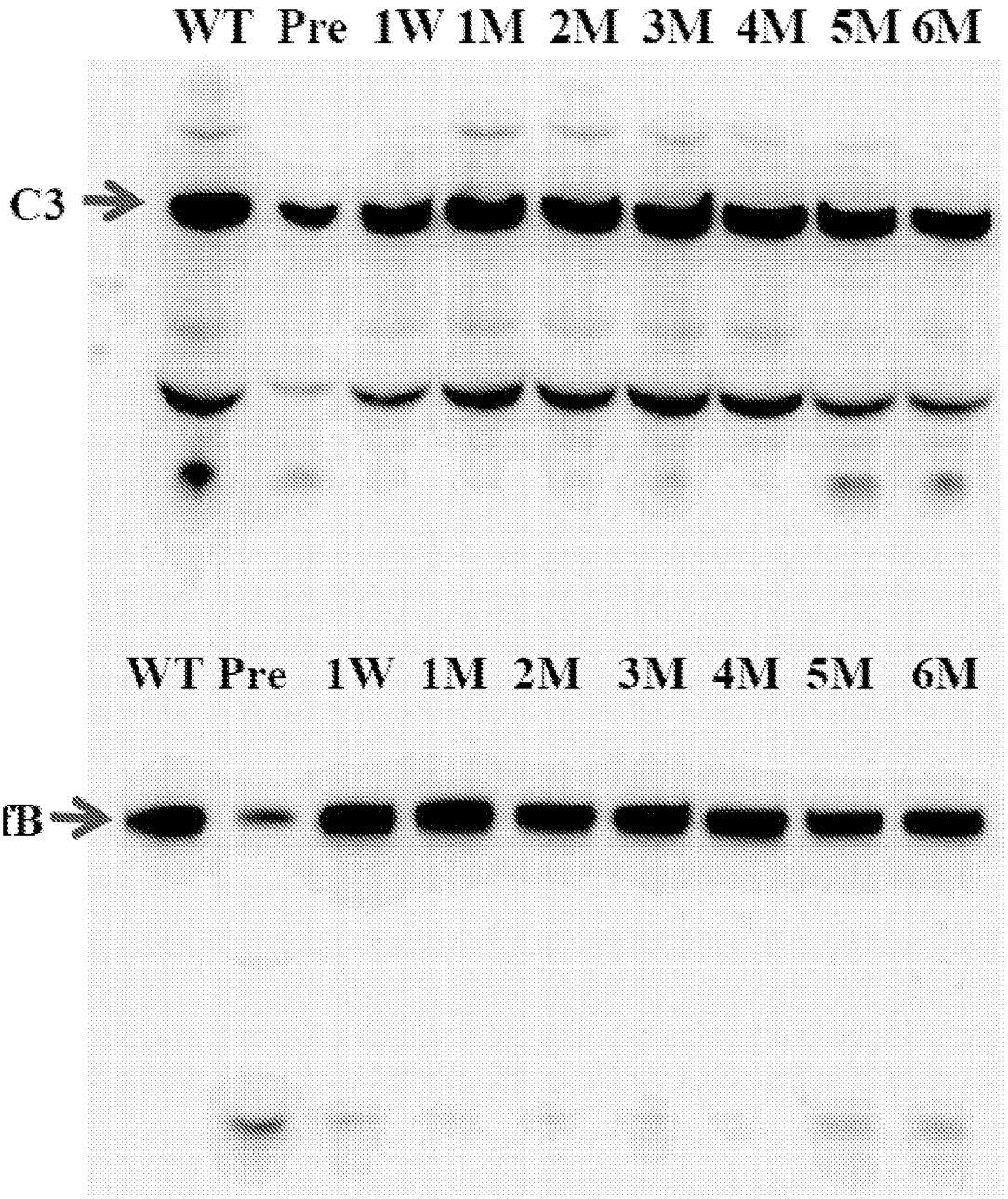

C3
fB
1 2 3 4 5 6 7 8 9
1: WT
2: M1 pre
3: M1 1W
4: M2 pre
5: M2 1W
6: M3 pre
7: M3 1W
8: M10 pr
9: M10 1W
Control AAV
AAV-mfH1-4.678.19-20
Control AAV
AAV-mfH1-4.678.19-20

Fig 24

Fig 25



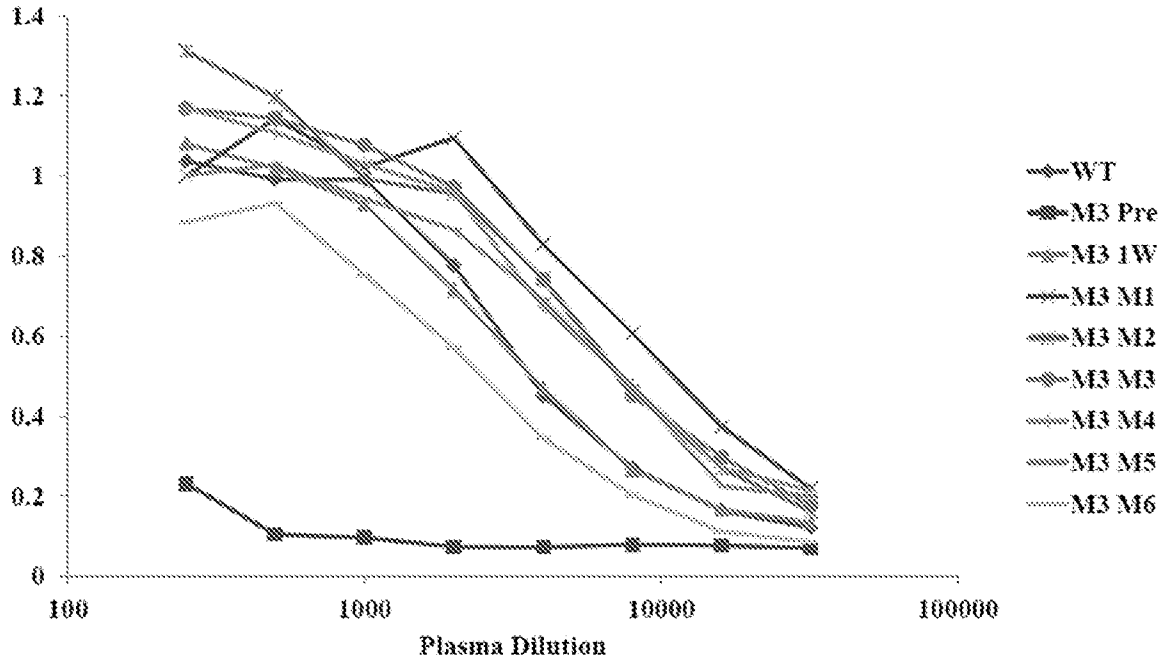

1.4
1.2
1
0.8
0.6
0.4
0.2
0
100
1000
10000
100000
Plasma Dilution
WT
M3 Pre
M3 1W
M3 M1
M3 M2
M3 M3
M3 M4
M3 M5
M3 M6

Fig 26

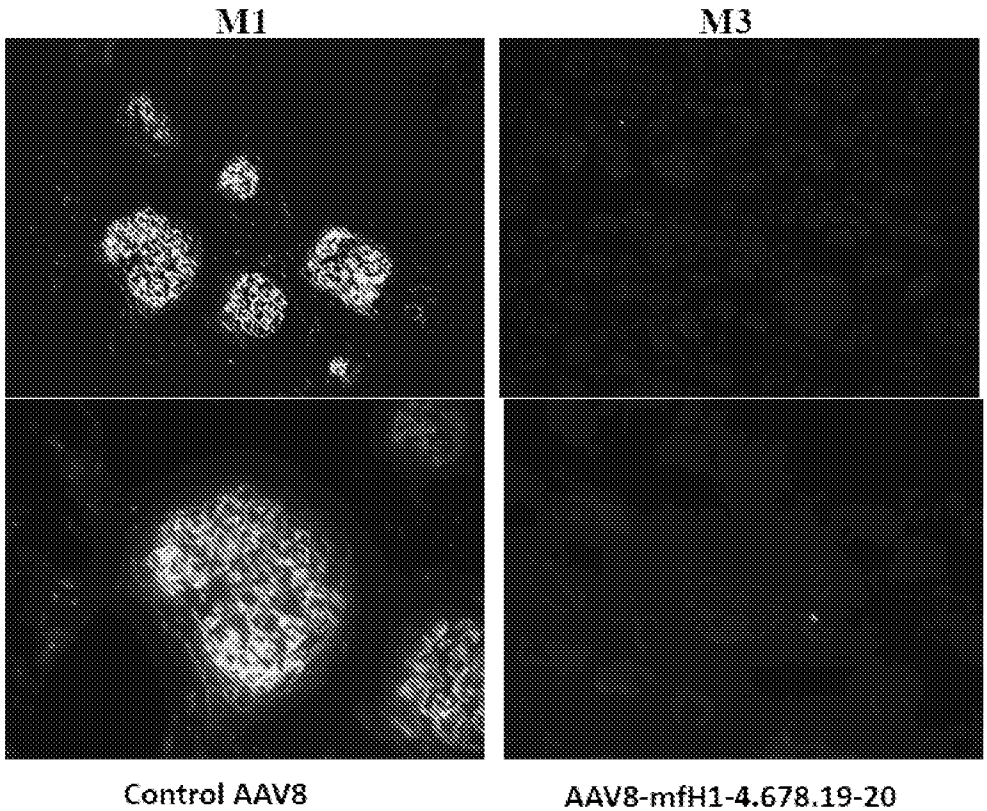

M1
M3
Control AAV8
AAV8-mfH1-4.678.19-20

FIG 27

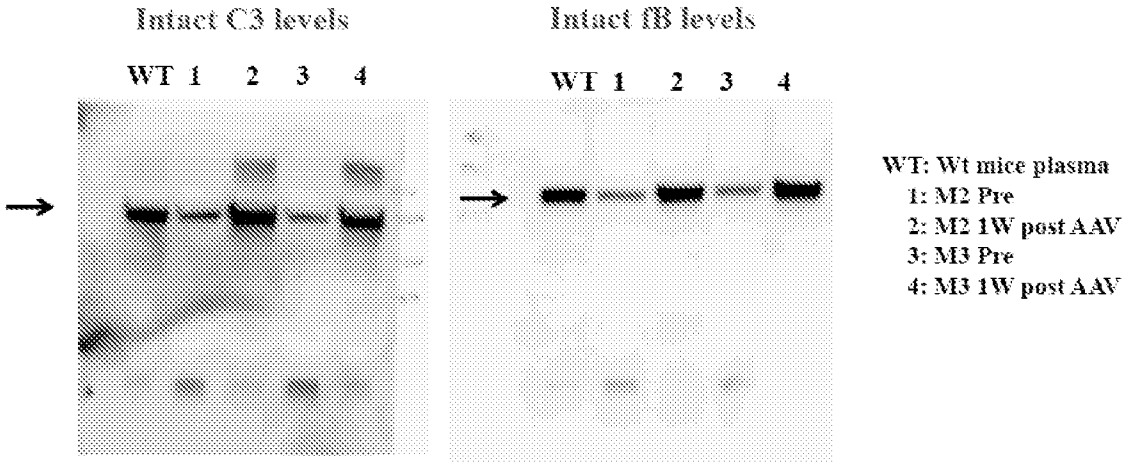


FIG 28A FIG 28B

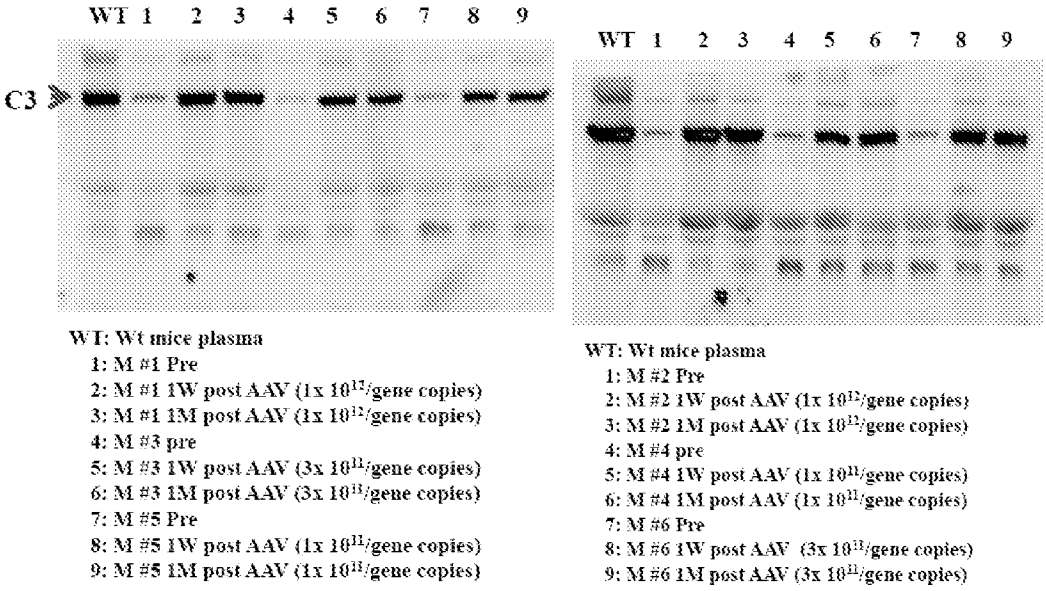


FIG 29A FIG 29B

Fig 30

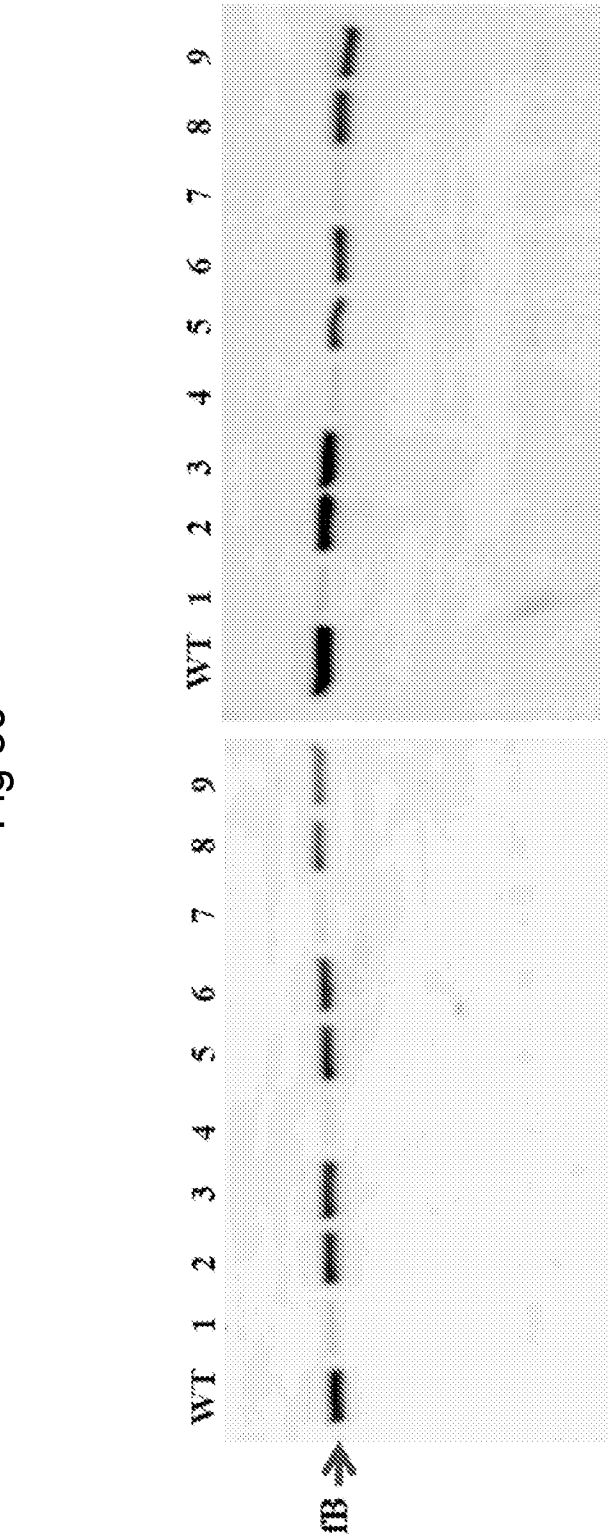

WT 1 2 3 4 5 6 7 8 9
fB
WT 1 2 3 4 5 6 7 8 9
WT: Wt mice plasma
1: M #1 Pre
2: M #1 1W post AAV (1x 10^12/gene copies)
3: M #1 1M post AAV (1x 10^12/gene copies)
4: M #3 pre
5: M #3 1W post AAV (3x 10^11/gene copies)
6: M #3 1M post AAV (3x 10^11/gene copies)
7: M #5 Pre
8: M #5 1W post AAV (1x 10^11/gene copies)
9: M #5 1M post AAV (1x 10^11/gene copies)
WT: Wt mice plasma
1: M #2 Pre
2: M #2 1W post AAV (1x 10^12/gene copies)
3: M #2 1M post AAV (1x 10^12/gene copies)
4: M #4 pre
5: M #4 1W post AAV (1x 10^11/gene copies)
6: M #4 1M post AAV (1x 10^11/gene copies)
7: M #6 Pre
8: M #6 1W post AAV (3x 10^11/gene copies)
9: M #6 1M post AAV (3x 10^11/gene copies)

FIG. 31

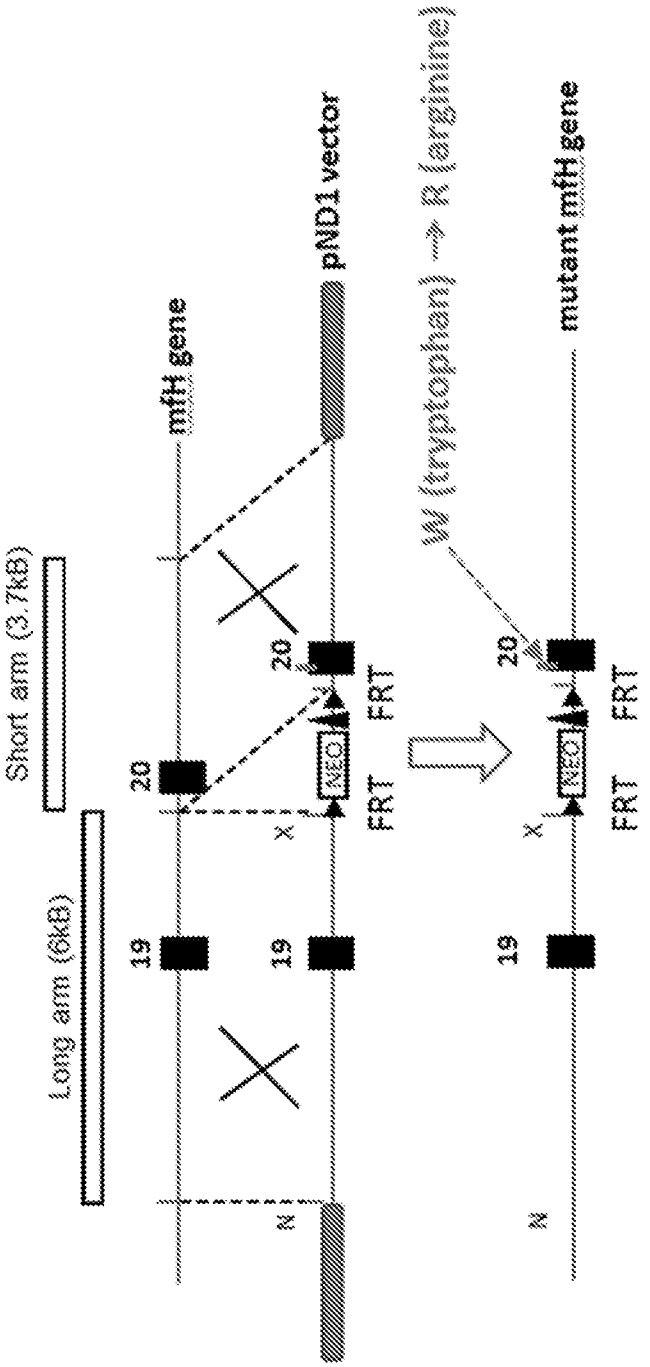
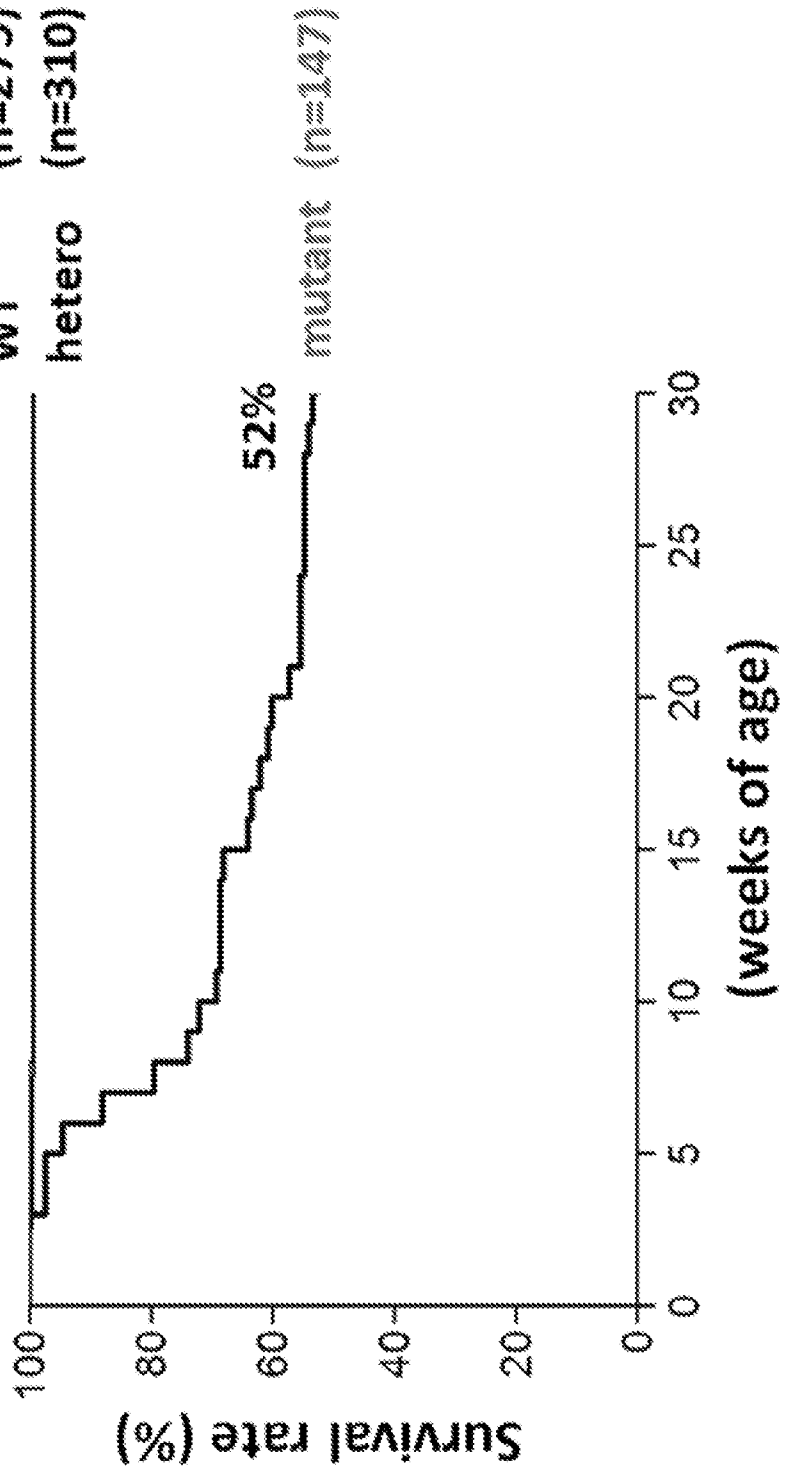

Short arm (3.7kB)
Long arm (6kB)
mfH gene
pND1 vector
20
19
NEO
FRT
FRT
W (tryptophan) → R (arginine)
mutant mfH gene

FIG 32


WT (n=275)
hetero (n=310)
mutant (n=147)
52%
Survival rate (%)
100
80
60
40
20
0
0
5
10
15
20
25
30
(weeks of age)

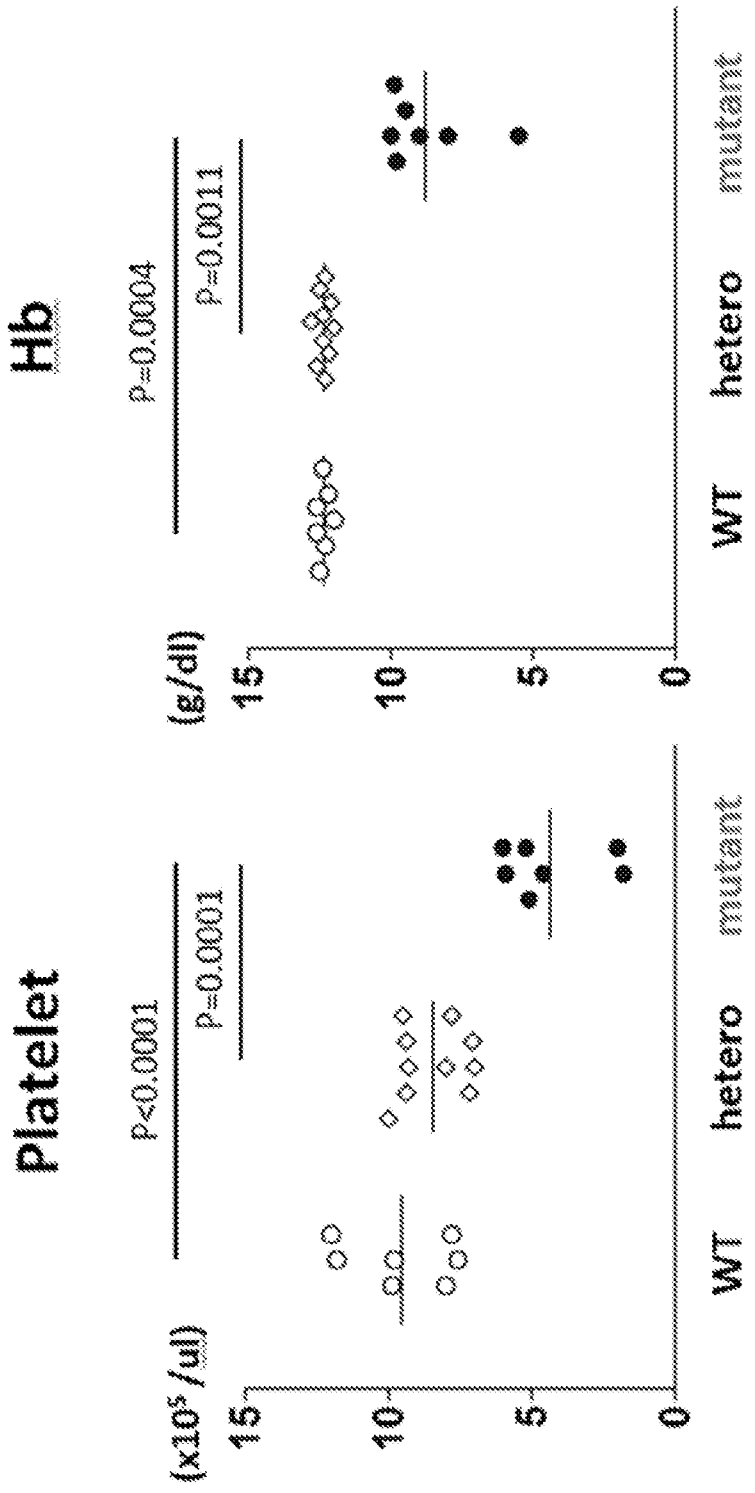


FIG 33A

FIG 33B

FIG. 34A

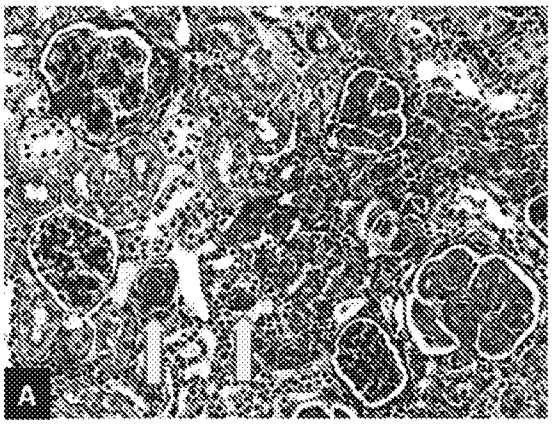


FIG. 34B

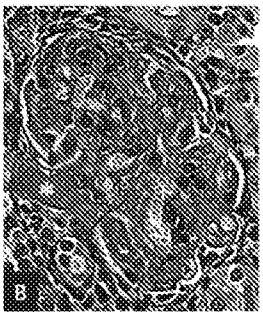


FIG. 34C

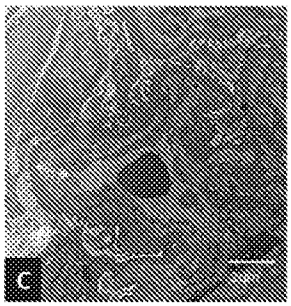


FIG 35A

FIG 35B

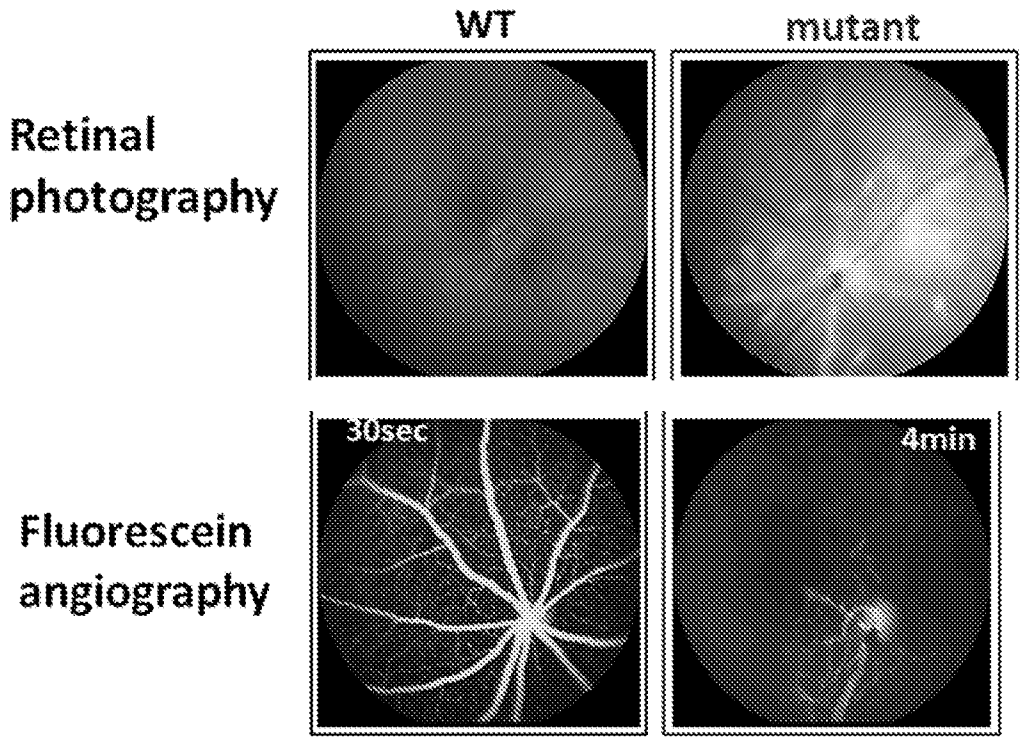


FIG 35C

FIG 35D

FIG 36

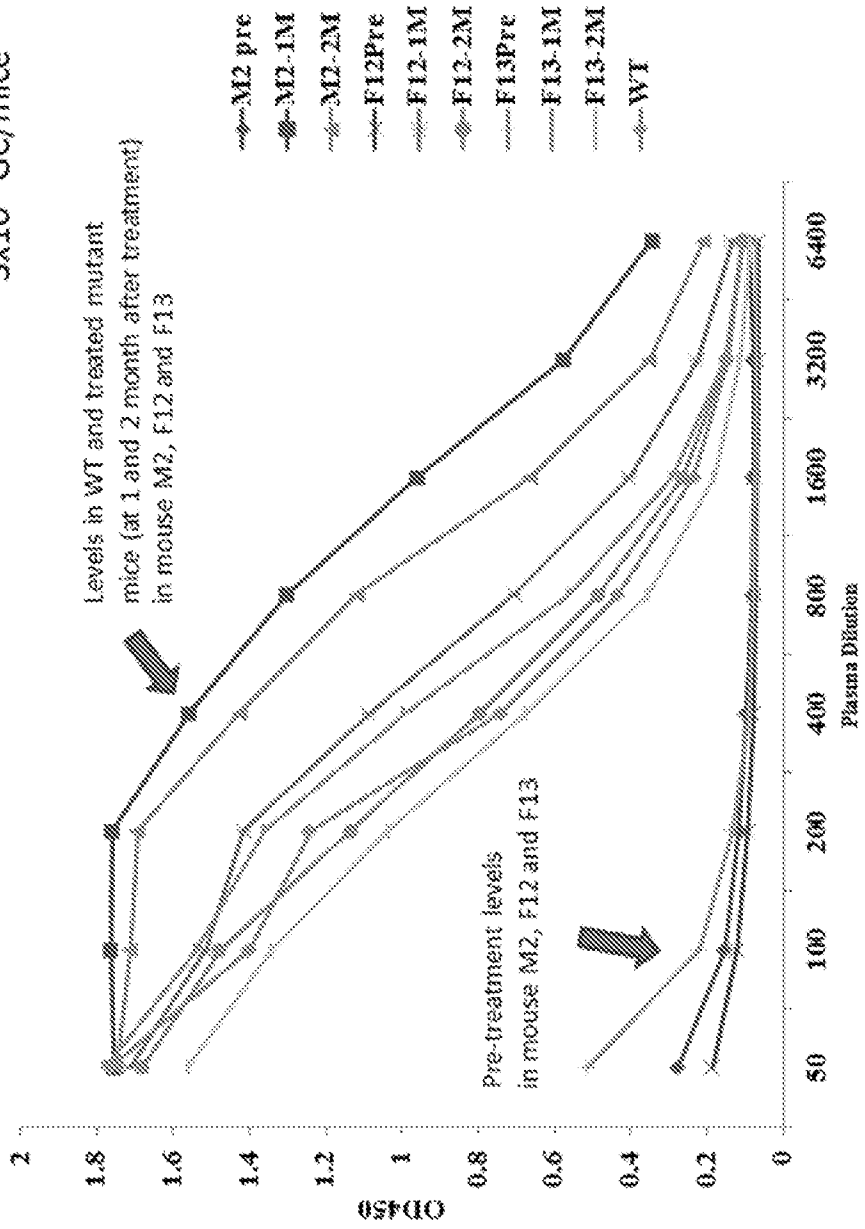

mfH1-4.678.19-20 expression in fH W1183R mice
3x10^11GC/mice
Levels in WT and treated mutant mice (at 1 and 2 month after treatment) in mouse M2, F12 and F13
Pre-treatment levels in mouse M2, F12 and F13
M2 pre
M2-1M
M2-2M
F12Pre
F12-1M
F12-2M
F13Pre
F13-1M
F13-2M
WT
OD450
Plasma Dilution
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
50
100
200
400
800
1600
3200
6400

FIG 37A

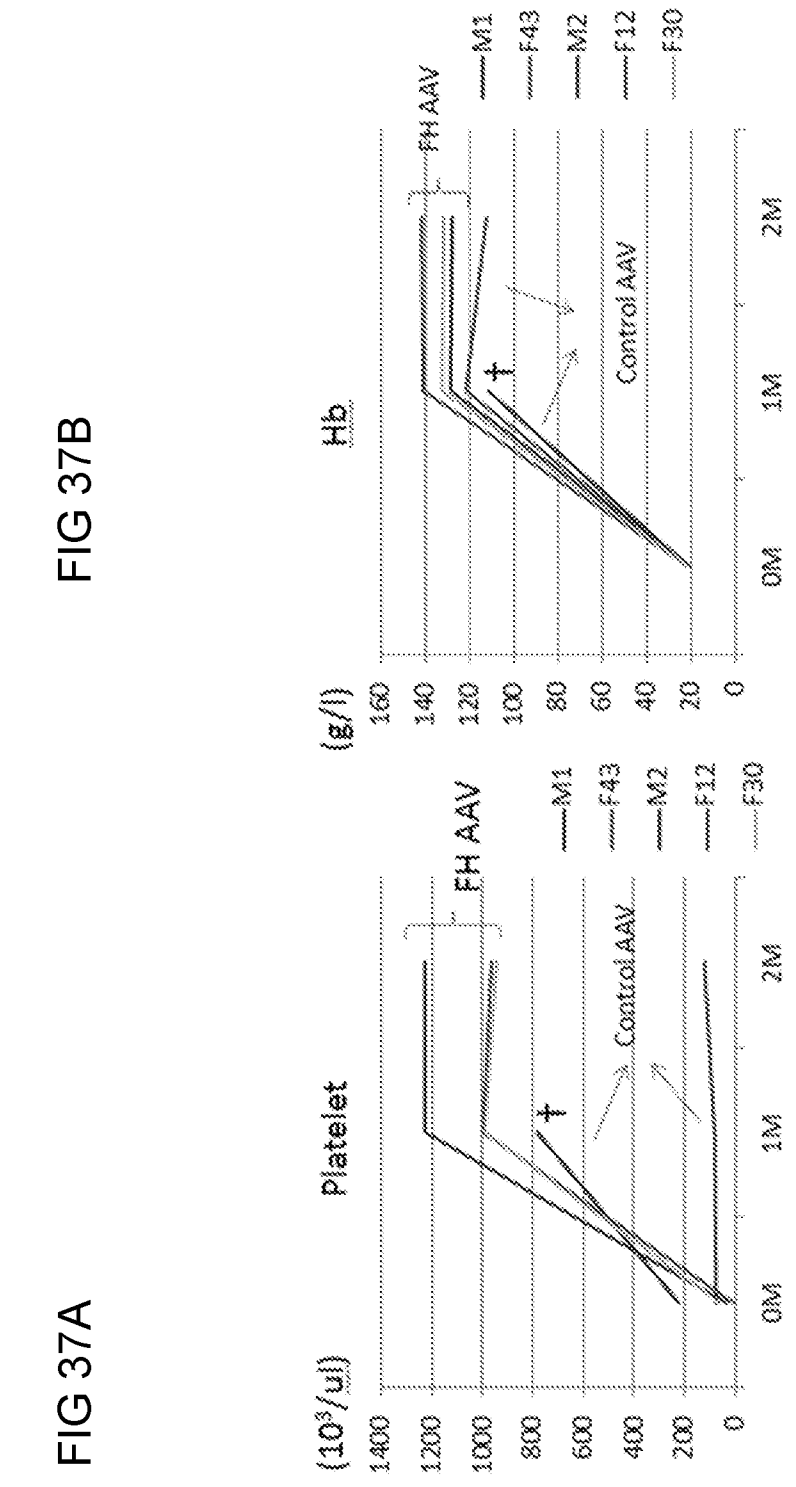


FIG 37B

control AAV : M1, F43
fH AAV : M2, F12, F30