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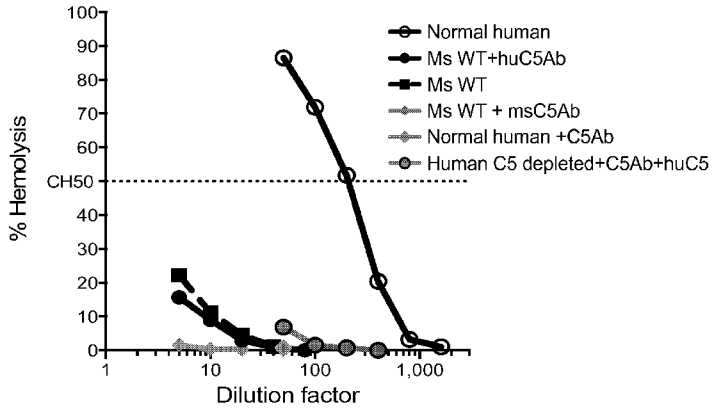
(54) Title: HUMANIZED C5 AND C3 ANIMALS

FIGURE 4



(57) Abstract: Non-human animals comprising a human or humanized C3 and/or C5 nucleic acid sequence are provided as well as methods for using the same to identify compounds capable of modulating the complement system. Non-human animals that comprise a replacement of the endogenous C5 gene and/or C3 gene with a human or humanized C5 gene and/or C3 gene, and methods for making and using the non-human animals, are described. Non-human animals comprising a human or humanized C5 gene under control of non-human C5 regulatory elements is also provided, including non-human animals that have a replacement of non-human C5-encoding sequence with human C5-encoding sequence at an endogenous non-human C5 locus. Non-human animals comprising a human or humanized C3 gene under control of non-human C3 regulatory elements is also provided, including non-human animals that have a replacement of non-human C3 protein-encoding sequence with human or humanized C3 protein-encoding sequence at an endogenous non-human C3 locus. Non-human animals comprising human or humanized C3 and/or C5 sequences, wherein the non-human animals are rodents, e.g., mice or rats, are provided.

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— *with amended claims (Art. 19(1))*

HUMANIZED C5 AND C3 ANIMALS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 61/988,581, filed May 5, 2014, and U.S. Provisional Patent Application No. 62/067,836, filed October 23, 2014, the disclosures of each of which are incorporated by reference herein in their entirety.

FIELD OF INVENTION

[0002] Disclosed herein are non-human animals comprising nucleic acid sequences encoding a C3 protein and/or a C5 protein that comprise a human sequence as well as transgenic non-human animals comprising a C3 and/or a C5 gene that is human in whole or in part. Also disclosed herein are non-human animals that express human or humanized C3 and/or C5 proteins and methods for using non-human animals comprising human or humanized C3 and/or C5 nucleic acid sequences.

BACKGROUND

[0003] Complement proteins C5 and C3 are therapeutic targets for treatment of a variety of human diseases, disorders and conditions that are associated with complement activation, for example, ocular inflammatory and retinal degenerative diseases. The evaluation of pharmacokinetics (PK) and pharmacodynamics (PD) of therapeutic molecules that specifically target human C5 or human C3 proteins are routinely performed in non-human animals, *e.g.*, rodents, *e.g.*, mice or rats. However, the PD of such therapeutic molecules cannot properly be determined in certain non-human animals because these therapeutic molecules do not target the endogenous C5 or C3 proteins.

[0004] Moreover, the evaluation of therapeutic efficacy of human-specific C5 or C3 protein antagonists various non-human animal models of diseases

associated with an activated complement system is problematic in non-human animals in which such species-specific antagonists do not interact with the endogenous C5 or C3 proteins.

[0005] Accordingly, there is a need for non-human animals, *e.g.*, rodents, *e.g.*, murine animals, *e.g.*, mice or rats, in which the C5 and/or C3 genes of the non-human animal are humanized in whole or in part or replaced (*e.g.*, at the endogenous non-human loci) with human C5 and/or C3 genes comprising sequences encoding human or humanized C5 and/or C3 proteins, respectively. There is a need for such humanized non-human animals that express human or humanized C5 and/or C3 proteins in serum at concentrations similar to that of C5 and/or C3 proteins, respectively, present in serum of an age-matched non-human animal, that expresses functional C5 and/or C3 proteins, but does not comprise the human or humanized C5 and/or C3 genes, respectively.

[0006] Throughout this specification, various patents, patent applications and other types of publications (*e.g.*, journal articles, electronic database entries, *etc.*) are referenced. The disclosure of all patents, patent applications, and other publications cited herein are hereby incorporated by reference in their entirety for all purposes.

SUMMARY

[0007] Non-human animals comprising nucleic acid sequences encoding a C3 and/or a C5 that comprise a human sequence are provided.

[0008] Transgenic non-human animals comprising a C3 and/or a C5 gene that is human in whole or in part are provided.

[0009] Non-human animals that express human or humanized C3 and/or C5 proteins are provided.

[00010] Non-human animals having a replacement (in whole or in part) of endogenous non-human animal C5 and/or C3 genes are provided.

[00011] Non-human animals comprising a C5 and/or C3 humanization (in

whole or in part) at an endogenous non-human C5 and/or C3 locus are provided.

[00012] Non-human animals are provided that have human or humanized C5 and/or C3 genes, wherein the non-human animals do not express endogenous C5 and/or C3 protein, and express human or humanized C5 and/or C3 protein, in serum at concentrations similar to that of C5 and/or C3 proteins, respectively, present in serum of an age-matched non-human animal that expresses functional endogenous C5 and/or C3 proteins, but does not comprise the replacement.

[00013] In one aspect, non-human animals comprising a human or humanized C3 and/or C5 nucleic acid sequence are provided.

[00014] In one aspect, genetically modified non-human animals are provided that comprise a replacement at an endogenous C5 and/or C3 locus of a gene encoding an endogenous C5 and/or C3 gene encoding a human or humanized C5 and/or C3 protein. Rodents, *e.g.*, mice or rats, are provided that comprise a replacement of an endogenous C5 gene, at an endogenous rodent C5 locus, with a human C5 gene, and/or comprise a replacement of an endogenous C3 gene, at an endogenous rodent C3 locus, with a human C3 gene. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00015] In one aspect, genetically modified rodents, *e.g.*, mice or rats, are provided comprising a replacement at an endogenous rodent C5 locus of a rodent gene encoding C5 protein with a human C5 gene encoding human or humanized C5 protein, wherein expression of the human C5 gene encoding human or humanized C5 protein is under control of rodent regulatory elements at the endogenous rodent C5 locus. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00016] In one embodiment, the human C5 gene encoding human or humanized C5 protein comprises exon 2 through exon 41 of the human C5 gene.

[00017] In one embodiment, the rodent is a mouse that is incapable of

expressing a mouse C5 protein.

[00018] In one embodiment, the rodent is a mouse that expresses a mouse C3 protein encoded by an endogenous mouse C3 gene.

[00019] In one embodiment, the rodent is mouse that expresses a human or humanized C3 protein.

[00020] In one embodiment, the rodent is a mouse that comprises a replacement at an endogenous mouse C3 locus of a mouse gene encoding C3 protein with a human C3 gene encoding a human or humanized C3 protein.

[00021] In one embodiment, expression of the human C3 gene encoding the human or humanized C3 protein is under control of mouse regulatory elements at the endogenous mouse C3 locus.

[00022] In one aspect, genetically modified rodents, *e.g.*, mice or rats, are provided comprising a replacement at an endogenous rodent C3 locus of a rodent gene encoding C3 protein with a human C3 gene encoding human or humanized C3 protein, wherein expression of the human C3 gene encoding human or humanized C3 protein is under control of rodent regulatory elements at the endogenous rodent C3 locus. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00023] In one embodiment, the human C3 gene encoding human or humanized C3 protein comprises exon 1 through exon 41 of the human C3 gene.

[00024] In one embodiment, the human C3 gene encoding human or humanized C3 protein comprises exon 2 through exon 41 of the human C3 gene.

[00025] In one embodiment, the rodent is a mouse that is incapable of expressing a mouse C3 protein.

[00026] In one embodiment, the rodent is a mouse that expresses a mouse C5 protein encoded by an endogenous mouse C5 gene.

[00027] In one embodiment, the rodent is mouse that expresses a human or humanized C5 protein.

[00028] In one embodiment, the rodent is a mouse that comprises a

replacement at an endogenous mouse C5 locus of a mouse gene encoding C5 protein with a human C5 gene encoding a human or humanized C5 protein.

[00029] In one embodiment, expression of the human C5 gene encoding the human or humanized C5 protein is under control of mouse regulatory elements at the endogenous mouse C5 locus.

[00030] In one aspect, genetically modified rodents, *e.g.*, a mouse or rat, are provided that express a human or humanized C5 protein, wherein the rodent that expresses a human or humanized C5 protein comprises a normal complement system, *e.g.*, the levels of complement proteins in the blood, plasma or serum of the rodent expressing human or humanized C5 protein are similar to the levels of complement proteins in the blood, plasma or serum of a rodent that expresses functional endogenous C5 protein. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00031] In one aspect, genetically modified rodents, *e.g.*, a mouse or rat, are provided that express C5 protein from a human C5 gene, wherein the rodent expresses human or humanized C5 protein in its serum. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00032] In one embodiment, the serum of the rodent that expresses a human or humanized C5 protein has approximately the same level of C5 protein as a rodent that expresses a functional, endogenous C5 protein, *e.g.*, a wild-type mouse or rat. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00033] In one embodiment, the mouse expresses human or humanized C5 protein in serum at a concentration of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190% or 200% of the level of C5 protein present in the serum of an age-matched mouse that expresses functional endogenous C5 protein, but does not comprise a replacement of an endogenous C5 gene, at an endogenous mouse C5 locus, with a human C5 gene.

[00034] In one embodiment, the mouse expresses human or humanized C5 protein in serum at a concentration of between about 10% and about 200%, between about 20% and about 150%, or between about 30% and about 100% of the level of mouse C5 protein present in the serum of an age-matched mouse that expresses functional endogenous C5 protein, but does not comprise a replacement of an endogenous C5 gene, at an endogenous mouse C5 locus, with a human C5 gene.

[00035] In one embodiment, the mouse expresses human or humanized C5 protein in serum at a concentration of between about 10 μg/ml and about 150 μg/ml, between about 10 μg/ml and about 125 μg/ml, or between about 15 μg/ml and about 100 μg/ml.

[00036] In one embodiment, the mouse expresses human or humanized C5 protein in serum at a concentration of at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125 or 150 μg/ml.

[00037] In one aspect, genetically modified rodents, *e.g.*, a mouse or rat, are provided that express a human or humanized C3 protein, wherein the rodent that expresses a human or humanized C3 protein comprises a normal complement system, *i.e.*, the levels of complement proteins in the blood, plasma or serum of the rodent expressing human or humanized C3 protein are similar to the levels of complement proteins in the blood, plasma or serum of a rodent that expresses functional endogenous C3 protein. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00038] In one aspect, genetically modified rodents, *e.g.*, a mouse or rat, are provided that express C3 protein from a human C3 gene, wherein the rodent expresses human or humanized C3 protein in its serum. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00039] In one embodiment, the serum of the rodent that expresses a human or humanized C3 protein has approximately the same level of C3 protein as a rodent that expresses a functional, endogenous C3 protein, *e.g.*, a wild-type

mouse or rat. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00040] In one embodiment, the mouse expresses human or humanized C3 protein (hC3) in serum at a concentration of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190% or 200% of the level of C3 protein present in the serum of an age-matched mouse that expresses functional endogenous C3 protein, but does not comprise a replacement of an endogenous C3 gene, at an endogenous mouse C3 locus, with a human C3 gene.

[00041] In one embodiment, the mouse expresses human C3 protein in serum at a concentration of between about 10% and about 200%, between about 20% and about 150%, or between about 30% and about 100% of the level of mouse C3 protein present in the serum of an age-matched mouse that expresses functional endogenous C3 protein, but does not comprise a replacement of an endogenous C3 gene, at an endogenous mouse C3 locus, with a human C3 gene.

[00042] In one embodiment, the mouse expresses human or humanized C3 protein in serum at a concentration of between about 100 μg/ml and about 1500 μg/ml, between about 200 μg/ml and about 1250 μg/ml, or between about 300 μg/ml and about 1000 μg/ml.

[00043] In one embodiment, the mouse expresses human or humanized C3 protein in serum at a concentration of at least about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1250 or 1500 μg/ml.

[00044] In one aspect, a genetically modified rodent is provided, comprising a human C5 gene comprising a replacement at an endogenous rodent C5 locus of a rodent gene encoding C5 protein with a human C5 gene encoding human or humanized C5 protein, wherein expression of the human C5 gene encoding human or humanized C5 protein is under control of rodent regulatory elements or sequences at the endogenous rodent C5 locus, and wherein the rodent further

comprises a human C3 gene comprising a replacement at an endogenous rodent C3 locus of a rodent gene encoding C3 protein with a human C3 gene encoding human or humanized C3 protein, wherein expression of the human C3 gene encoding human or humanized C3 protein is under control of rodent regulatory elements or sequences at the endogenous rodent C3 locus. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00045] In one embodiment, the mouse is incapable of expressing a mouse C5 protein and incapable of expressing a mouse C3 protein.

[00046] In one embodiment, the rodent regulatory elements or sequences at the endogenous rodent C5 locus and/or rodent C3 locus are from a mouse or a rat.

[00047] In one embodiment, the rodent regulatory elements or sequences are endogenous rodent regulatory elements or sequences at the rodent C5 locus and/or rodent C3 locus are from a mouse or a rat.

[00048] In one aspect, a non-human animal, *e.g.*, a rodent, *e.g.*, a mouse or rat, is provided that expresses human or humanized C5 and/or C3 proteins, wherein the non-human animal expresses human or humanized C5 and/or C3 proteins from an endogenous non-human C5 locus and/or an endogenous non-human C3 locus. In an embodiment, the non-human animal is a rodent. In an embodiment, the rodent is a mouse. In an embodiment, the rodent is a rat.

[00049] In one aspect, a genetically modified mouse is provided that expresses human or humanized C5 protein from an endogenous mouse C5 locus, wherein the endogenous mouse C5 gene has been replaced, in whole or in part, with a human C5 gene.

[00050] In one embodiment, about 75.8 kb at the endogenous mouse C5 locus, including exons 2 through 42 and a 3' untranslated sequence, is deleted and replaced with about 97 kb of human C5 gene sequence comprising exons 2 through 41 of the human C5 gene. In a specific embodiment, the human C5 gene comprises exons 2 through 42 of the human C5 gene of human BAC CTD-

2559l19. In a specific embodiment, the C5 gene comprises mouse C5 exon 1 and human C5 exons 2 through 42.

[00051] In one aspect, a genetically modified mouse is provided that comprises a nucleotide sequence encoding a human or humanized C5 protein, wherein the nucleotide sequence encoding the human or humanized C5 protein replaces, in whole or in part, an endogenous nucleotide sequence encoding an endogenous mouse C5 protein.

[00052] In one aspect, a genetically modified mouse is provided that expresses human or humanized C3 protein from an endogenous mouse C3 locus, wherein the endogenous mouse C3 gene has been replaced, in whole or in part, with a human C3 gene.

[00053] In one embodiment, a portion of the endogenous mouse C3 locus, including 5' regulatory elements upstream of exon 1 through exon 41, is deleted and replaced with a human C3 gene sequence comprising 5' regulatory elements upstream of exon 1 through exon 41 of the human C3 gene. In a specific embodiment, the human C3 gene comprises the entire human C3 coding region.

[00054] In one embodiment, a portion of the endogenous mouse C3 locus, including a portion of intron 1 and exons 2 through 41, is deleted and replaced with a human C3 gene sequence comprising a portion of intron 1 and exons 2 through exon 41 of the human C3 gene. In a specific embodiment, the C3 gene comprises mouse C3 exon 1 and human C3 exons 2 through 41.

[00055] In one aspect, a method is provided for making a humanized C5 rodent, comprising replacing a rodent C5 gene sequence encoding rodent C5 protein with a human C5 gene sequence comprising one or more exons of the human C5 gene sequence encoding human or humanized C5 protein. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00056] In one embodiment, the replacement is at an endogenous rodent C5 locus and the human C5 gene sequence comprising one or more exons of the human C5 gene sequence encoding human or humanized C5 protein is

operably linked to rodent regulatory elements or sequences at the endogenous rodent C5 locus. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00057] In one embodiment, the rodent regulatory elements or sequences are derived from a mouse. In one embodiment, the rodent regulatory elements or sequences are derived from a rat.

[00058] In one embodiment, the rodent regulatory elements or sequences are endogenous rodent regulatory elements or sequences at the rodent C5 locus. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00059] In one embodiment, the human C5 gene sequence replacing the rodent C5 gene sequence comprises at least one exon of the human C5 gene sequence. In other embodiments, the human C5 gene sequence replacing the rodent C5 gene sequence comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 exons of the human C5 gene sequence. In one embodiment, the human C5 gene sequence replacing the rodent C5 gene sequence comprises all 41 exons of the human C5 gene sequence. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00060] In one embodiment, the replacement is at an endogenous rodent C5 locus and the human C5 gene sequence comprising one or more exons of the human C5 gene sequence encoding human or humanized C5 protein is operably linked endogenous rodent regulatory elements or sequences at the endogenous rodent C5 locus.

[00061] In one aspect, a method is provided for making a humanized C5 mouse, comprising replacing a mouse C5 gene sequence encoding mouse C5 protein with a human C5 gene sequence encoding human or humanized C5 protein.

[00062] In one embodiment, the replacement is at an endogenous mouse

C5 locus, and the human C5 gene encoding human or humanized C5 protein is operably linked to mouse regulatory elements or sequences at the endogenous mouse C5 locus.

[00063] In one embodiment, the replacement is at an endogenous mouse C5 locus, and the human C5 gene encoding human or humanized C5 protein is operably linked to endogenous mouse regulatory elements or sequences at the endogenous mouse C5 locus.

[00064] In one aspect, a method is provided for making a humanized C3 rodent, comprising replacing a rodent C3 gene sequence encoding rodent C3 protein with a human C3 gene sequence comprising one or more exons of the human C3 gene sequence encoding human or humanized C3 protein. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00065] In one embodiment, the replacement is at an endogenous rodent C3 locus and the human C3 gene sequence comprising one or more exons of the human C3 gene sequence encoding human or humanized C3 protein is operably linked to rodent regulatory elements or sequences at the endogenous rodent C3 locus. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00066] In one embodiment, the rodent regulatory elements or sequences are derived from a mouse. In one embodiment, the rodent regulatory elements or sequences are derived from a rat.

[00067] In one embodiment, the rodent regulatory elements or sequences are endogenous rodent regulatory elements or sequences at the rodent C3 locus. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00068] In one embodiment, the human C3 gene sequence replacing the rodent C3 gene sequence comprises at least one exon of the human C3 gene sequence. In other embodiments, the human C3 gene sequence replacing the rodent C3 gene sequence comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13,

14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 exons of the human C3 gene sequence. In one embodiment, the human C3 gene sequence replacing the rodent C5 gene sequence comprises all 41 exons of the human C3 gene sequence. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00069] In one embodiment, the replacement is at an endogenous rodent C3 locus and the human C3 gene sequence comprising one or more exons of the human C3 gene sequence encoding human or humanized C3 protein is operably linked endogenous rodent regulatory elements or sequences at the endogenous rodent C3 locus.

[00070] In one aspect, a method is provided for making a humanized C3 mouse, comprising replacing a mouse C3 gene sequence encoding mouse C3 protein with a human C3 gene sequence encoding human or humanized C3 protein.

[00071] In one embodiment, the replacement is at an endogenous mouse C3 locus, and the human C3 gene encoding human or humanized C3 protein is operably linked to endogenous mouse regulatory elements or sequences at the endogenous mouse C3 locus.

[00072] In one embodiment, the replacement is at an endogenous mouse C3 locus, and the human C3 gene encoding human or humanized C3 protein is operably linked to mouse regulatory elements or sequences at the endogenous mouse C3 locus.

[00073] In various aspects, the genetically modified non-human animals, *e.g.*, rodents, *e.g.*, mice or rats, described herein comprise the genetic modifications in their germ-line.

[00074] In one aspect, a non-human animal, *e.g.*, rodent, *e.g.*, a mouse or rat, embryo comprising a genetic modification as described herein is provided.

[00075] In one aspect, a non-human animal, *e.g.*, rodent, *e.g.* a mouse or rat, host embryo is provided that comprises a donor cell that comprises a genetic

modification as described herein.

[00076] In one aspect, a pluripotent or totipotent non-human animal, *e.g.*, rodent, *e.g.*, mouse or rat, cell comprising a genetic modification as described herein is provided. In one embodiment, the cell is a rodent cell. In one embodiment, the cell is a mouse cell. In one embodiment, the cell is a rodent ES cell. In one embodiment, the cell is a mouse ES cell.

[00077] In one aspect, a non-human animal, *e.g.*, rodent, *e.g.*, mouse or rat, egg is provided, wherein the non-human animal egg comprises an ectopic non-human animal chromosome, wherein the ectopic non-human animal chromosome comprises a genetic modification as described herein. In one embodiment, the non-human animal is a rodent. In one embodiment, the rodent is a mouse. In one embodiment, the rodent is a rat.

[00078] In one aspect, the mouse embryo, egg, or cell that is genetically modified to comprise a human C5 gene or human C3 gene is of a mouse that is of a C57BL strain selected from C57BL/A, C57BL/An, C57BL/GrFa, C57BL/KaLwN, C57BL/6, C57BL/6J, C57BL/6ByJ, C57BL/6NJ, C57BL/10, C57BL/10ScSn, C57BL/10Cr, and C57BL/Ola. In another embodiment, the mouse is a 129 strain selected from the group consisting of a strain that is 129P1, 129P2, 129P3, 129X1, 129S1 (*e.g.*, 129S1/SV, 129S1/SvIm), 129S2, 129S4, 129S5, 129S9/SvEvH, 129S6 (129/SvEvTac), 129S7, 129S8, 129T1, 129T2 (*see*, *e.g.*, Festing et al. (1999) Revised nomenclature for strain 129 mice, Mammalian Genome 10:836, see also, Auerbach et al (2000) Establishment and Chimera Analysis of 129/SvEv- and C57BL/6-Derived Mouse Embryonic Stem Cell Lines). In a specific embodiment, the genetically modified mouse is a mix of an aforementioned 129 strain and an aforementioned C57BL/6 strain. In another specific embodiment, the mouse is a mix of aforementioned 129 strains, or a mix of aforementioned BL/6 strains. In a specific embodiment, the 129 strain of the mix is a 129S6 (129/SvEvTac) strain. In another embodiment, the mouse is a BALB strain, *e.g.*, BALB/c strain. In yet another embodiment, the mouse is a mix of a BALB strain and another aforementioned strain. In one embodiment, the

mouse is Swiss or Swiss Webster mouse.

[00079] In a further aspect, provided herein are methods for identifying a compound capable of modulating complement activation comprising administering the compound to any of the non-human animals, *e.g.*, a rodent, *e.g.*, a mouse or rat disclosed and described herein; and assaying if complement activation in the rodent is modulated, thereby identifying a compound capable of modulating complement activation. In one embodiment, the compound is a small molecule chemical compound, an antibody, a protein, an inhibitory nucleic acid, or any combination thereof. In another embodiment of any of the embodiments disclosed herein, the compound modulates complement activation by increasing complement activity. In another embodiment of any of the embodiments disclosed herein, the compound modulates complement activation by decreasing complement activity. In another embodiment of any of the embodiments disclosed herein, the serum of the rodent is assayed to determine if complement activation in the rodent is modulated. In a further embodiment, the assay is a CH_{50} complement screening assay.

[00080] Each of the aspects and embodiments described herein are capable of being used together, unless excluded either explicitly or clearly from the context of the embodiment or aspect.

BRIEF DESCRIPTION OF THE DRAWINGS

[00081] Figure 1 provides an illustration, not to scale, of the mouse (top) and human (bottom) C5 genomic loci. The regions of the mouse and human C5 genes that are deleted and replaced, respectively, to generate humanized C5 mice are indicated.

[00082] Figure 2 provides an illustration, not to scale, of the mouse (mC3) and humanized (hC3) C3 genomic loci. (A) The mouse C3 gene spanning the 5' regulatory elements and the coding region from exon 1 through exon 41 are deleted and replaced by the 5' regulatory elements and the coding region from

exon 1 through exon 41 of the human C3 gene and a loxP site, as indicated by a bold line and an arrow, respectively. (B) The mouse C3 gene spanning a portion of intron 1 and the coding region from exon 2 through exon 41 are deleted and replaced by a portion of intron 1 and the coding region from exon 2 through exon 41 of the human C5 gene and a loxP site, as indicated by a bold line and an arrow, respectively.

[00083] Figure 3 shows that mouse complement has lower activity in a hemolytic assay than human complement.

[00084] Figure 4 shows that complement hemolytic activity is blocked by species-specific anti-C5 monoclonal antibodies in a species-specific manner.

[00085] Figure 5 shows that humanized C5 mice are functional mouse C5 knockouts. (A) Addition of human C5 or C3 protein reconstitutes hemolytic activity in C5 or C3-depleted human serum, respectively. (B) Mouse, but not human, C5 protein reconstitutes hemolytic activity in $C5^{-/-}$ and humanized C5 mouse sera. (C) Levels of human C5 protein in humanized C5 mouse serum is three times lower than in human serum. (D) Pre-dose complement C5 levels in humanized C5 mice vary between males and females. A range of human C5, from 20-100 µg/mL is present in humanized C5 mouse serum.

[00086] Figure 6 shows that addition of human C3 and C5 proteins, but not human C5 protein alone, reconstitutes hemolytic activity in $C5^{-/-}$ mouse serum.

[00087] Figure 7 shows that humanized C5 mouse serum requires addition of human C3 protein to achieve wild-type levels of mouse hemolytic activity.

[00088] Figure 8 shows the results of a hemolysis assay performed on serum samples from humanized C5 mice exposed to the anti-human C5 antibody (C5Ab) using 400 µg/mL human C3 protein (A) and 800 µg/mL human C3 protein (B).

[00089] Figure 9 shows the results of a pharmacokinetic assay measuring anti-human C5 antibody levels as a function of time post-injection for all animals in the study (A) and for female (top) and male (bottom) animals (B).

[00090] Figure 10A shows that adding human C3 protein to wild type mouse serum can enhance hemolytic function but not to the same extent observed in normal human serum (for each data point from mice, serum was mixed from 3 animals). Figure 10B shows that hemolytic function can be restored in humanized $C5^{hu/hu}$ mice when supplemented with human C3 protein.

[00091] Figure 11 shows that adding human C3 protein to serum derived from C3 knockout mice can rescue hemolytic function (for each data point from mice, serum was mixed from 3 animals).

[00092] Figure 12 shows that adding human C3 protein only to C5 knockout mouse serum cannot rescue hemolytic function (for each data point from mice, serum was mixed from 3 animals).

DETAILED DESCRIPTION

[00093] The complement system is an essential component of the innate immune system and plays a critical role as a defense mechanism against invading pathogens, primes adaptive immune responses, and helps remove immune complexes and apoptotic cells. While the complement system plays a critical role in many protective immune functions, complement activation is a significant mediator of tissue damage in a wide range of autoimmune and inflammatory disease processes.

[00094] The invention described herein provides, *inter alia,* non-human animals that express human or humanized C5 and/or C3 proteins in serum at concentrations similar to wild type expression of endogenous C5 and/or C3 proteins in non-human animals. The successful engineering of non-human animals capable of expressing human or humanized C5 and/or C3 proteins provides a needed and useful tool for recapitulating the human complement system in laboratory animals amenable to large scale and high-throughput drug screening assays. Also provided herein are methods for using the non-human animals (such as rodents) described herein for identifying compounds capable of

modulating complement activation. The methods are based, in part, on the inventors' discovery that while complement activity of C5 protein is species-specific (*i.e.*, human C5 protein is unable to substitute for mouse C5 protein), the complement activity of human C3 protein is able to substitute for mouse C3 protein in a mouse serum complement activity.

General Techniques

[00095] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, cell biology, biochemistry, nucleic acid chemistry, and immunology, which are well known to those skilled in the art. Such techniques are explained fully in the literature, such as, *Molecular Cloning: A Laboratory Manual*, fourth edition (Sambrook et al., 2012) and *Molecular Cloning: A Laboratory Manual*, third edition (Sambrook and Russel, 2001), (jointly referred to herein as "Sambrook"); *Current Protocols in Molecular Biology* (F.M. Ausubel et al., eds., 1987, including supplements through 2014); *PCR: The Polymerase Chain Reaction*, (Mullis et al., eds., 1994); *Antibodies: A Laboratory Manual*, Second edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (Greenfield, ed., 2014), Beaucage et al. eds., *Current Protocols in Nucleic Acid Chemistry*, John Wiley & Sons, Inc., New York, 2000, (including supplements through 2014) and *Gene Transfer and Expression in Mammalian Cells* (Makrides, ed., Elsevier Sciences B.V., Amsterdam, 2003).

Definitions

[00096] As used herein, the term "protein" includes polypeptides, peptides, fragments of polypeptides, and fusion polypeptides.

[00097] As used herein, a "nucleic acid" refers to two or more deoxyribonucleotides and/or ribonucleotides covalently joined together in either single or double-stranded form.

[00098] By "operably linked" is meant a functional linkage between a nucleic acid expression control sequence (such as a promoter) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

[00099] The term "replacement" in reference to gene replacement refers to placing exogenous genetic material at an endogenous genetic locus, thereby replacing all or a portion of the endogenous gene with an orthologous or homologous nucleic acid sequence. In one instance, an endogenous non-human gene or fragment thereof is replaced with a corresponding human gene or fragment thereof. A corresponding human gene or fragment thereof is a human gene or fragment that is an ortholog of, a homolog of, or is substantially identical or the same in structure and/or function, as the endogenous non-human gene or fragment thereof that is replaced. As demonstrated in the Examples below, nucleotide sequences of endogenous non-human (for example rodent, such as, mouse) C3 and/or C5 gene loci were replaced by nucleotide sequences corresponding to human C3 and/or C5 gene loci. In another embodiment, a gene replacement can occur when an endogenous gene is deleted or rendered nonfunctional (such as by the insertion of a missense mutation or a premature stop codon) and a corresponding human gene or fragment thereof is inserted into the germline at a separate location.

[000100] The term "humanized" as used in the phrases "humanized C3 allele" or "humanized C5 allele," or "humanized C3 gene," or "humanized C5 gene" includes, but is not limited to, embodiments wherein all or a portion of an endogenous non-human C3 and/or C5 gene or allele is replaced by a corresponding portion of the human C3 and/or C5 gene or allele. For example, in some embodiments, the term "humanized" refers to the complete replacement of the coding region (e.g., the exons) of the endogenous non-human C3 and/or C5 gene or allele with the corresponding coding region of the human C3 and/or C5 gene or allele, while the endogenous non-coding region(s) (such as, but not

limited to, the promoter, the 5' and/or 3' untranslated region(s), enhancer elements, *etc.*) of the non-human animal is not replaced. In some embodiments, the non-human animal is a rodent, such as a rat or mouse.

[000101] A "humanized protein" includes, but is not limited to, embodiments wherein all or a portion of the encoded endogenous non-human C3 and/or C5 protein is replaced by the corresponding portion of the human C3 and/or C5 protein. In some embodiments, a "humanized protein" can be encoded by a humanized C3 and/or C5 gene or allele but still encode a fully human C3 and/or C5 protein (such as, but not limited to, the situation wherein all of the coding regions (e.g., the exons) of the endogenous non-human C3 and/or C5 gene or allele are replaced by the corresponding coding regions of the human C3 and/or C5 gene or allele but the endogenous non-coding region(s) (such as, but not limited to, the promoter, the 5' and/or 3' untranslated region(s), enhancer elements, *etc.*) of the non-human animal is not replaced). In some embodiments, the non-human animal is a rodent, such as a rat or mouse.

[000102] "Modulates," as used herein, refers to the ability of a compound to alter the activity of the complement system. In one embodiment, modulation of the complement system by a compound refers to decreasing complement activation in a subject (for example, a rodent, such as a mouse). In another embodiment, modulation of the complement system by a compound refers to increasing complement-mediated activity in a subject.

[000103] As used herein, the term "rodent" refers to any member of the order Rodentia. Examples of rodents include, without limitation, mice, rats, squirrels, prairie dogs, porcupines, beavers, guinea pigs, and hamsters. In one embodiment, a rodent is a rat. In another embodiment, a rodent is a mouse.

[000104] Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains.

[000105] As used herein, the singular terms "a," "an," and "the" include the plural reference unless the context clearly indicates otherwise.

[000106] It is intended that every maximum numerical limitation given throughout this specification includes every lower numerical limitation, as if such lower numerical limitations were expressly written herein. Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

Complement System

[000107] Complement, an essential component of the immune system, consists of more than 30 serum and cellular proteins that are involved in two linked biochemical cascades, the classical and alternative pathways. Complement functions to assist the immune system to destroy invading microorganisms and maintain tissue homeostasis.

[000108] However, excessive or unregulated activation of complement contributes to tissue damage, and is associated with a variety of variety of human diseases, disorders and conditions that are associated with complement activation, for example, ocular inflammatory and retinal degenerative diseases (*see* Makrides (1998) Therapeutic inhibition of the complement system, *Pharmacological Revie*ws 50(1):59-87; and Mollnes et al. (2006) Strategies of therapeutic complement inhibition, *Molecular Immunology* 43;107-121). Other diseases or conditions known to be associated with aberrant complement activation include, without limitation, allergic asthma and the accompanying airway inflammation and airway hyperresponsiveness ("AHR"), chronic obstructive pulmonary disease ("COPD"), allergic bronchopulmonary aspergillosis, hypersensitivity pneumonia, eosinophilic pneumonia, emphysema, bronchitis, allergic bronchitis, bronchiectasis, cystic fibrosis, tuberculosis,

hypersensitivity pneumonitis, occupational asthma, sarcoid, reactive airway disease syndrome, interstitial lung disease, hyper-eosinophilic syndrome, rhinitis, sinusitis, exercise-induced asthma, pollution-induced asthma, cough variant asthma, parasitic lung disease, respiratory syncytial virus ("RSV") infection, parainfluenza virus ("PIV") infection, rhinovirus ("RV") infection and adenovirus infection, and ischemia-reperfusion injury (*see, e.g.,* U.S. Patent Application Publication No. 2005/0260198, which is incorporated herein by reference).

Complement C5 Gene and Protein

[000109] The C5 gene encodes the serum complement C5 protein, which plays an important role in inflammatory and cell killing processes. Mature C5 protein is comprised of α and β chains that are linked by a disulfide bond. The C5 protein is cleaved by a convertase, resulting in an activation peptide, C5a, an anaphylatoxin derived from the α polypeptide, which is largely pro-inflammatory, and C5b, a macromolecular cleavage product derived from the β polypeptide, which forms a complex with other complement components to form a membrane attack complex (MAC), which is involved in osmotic lysis of target cells.

[000110] *Human C5.* Official symbol: ***C5***; NCBI Gene ID: 727; Primary source: HGNC:1331; RefSeq mRNA ID: NM_001735.2; UniProt ID: P01031; Genomic assembly: GRCh38; Location: chr9:120,952,335-121,050,275 – strand.

[000111] The human C5 gene is located on chromosome 9, at 9q33-q34. The human C5 gene has 41 exons and encodes a precursor polypeptide of 1676 amino acids in length, including an 18 amino acid signal peptide, a 655 amino acid β chain and a 999 amino acid α chain. During complement activation the α chain is cleaved, thereby generating a 74 amino acid C5a, which is a potent pro-inflammatory anaphylatoxin.

[000112] C5 deficiency in humans is associated with increased susceptibility to severe recurrent infections.

[000113] The C5 gene is conserved between several species, including primates, *e.g.*, chimpanzee, Rhesus monkey, other mammals, *e.g.*, dog, cow,

rodent, *e.g.*, mouse, chicken, zebrafish and frog.

[000114] *Mouse C5*. Official symbol: ***Hc***; NCBI Gene ID: 15139; Primary source: MGI:96031; RefSeq mRNA ID: NM_010406.2; UniProt ID: P06684; Genomic assembly: GRCm38; Location: chr2:34,983,331-35,061,449 – strand.

[000115] The mouse C5 gene is located on chromosome 2, at 2 23.22 cM. The mouse C5 gene has 42 exons and encodes a precursor polypeptide of 1680 amino acids in length, including an 18 amino acid signal peptide, a 656 amino acid β chain and a 1002 amino acid α chain. During complement activation the α chain is cleaved, thereby generating a 77 amino acid C5a, which is a potent pro-inflammatory anaphylatoxin.

[000116] C5 deficiency is observed in several common strains of laboratory mice which have a common 2 base pair deletion near the 5' end of the cDNA that results in inability of C5 to be secreted; thus, these mice are functionally C5 deficient, *i.e.*, C5 knockouts (6 such strains are A/HeJ, AKR/J, DBA/2J, NZB/B1NJ, SWR/J, and B10.D2/oSnJ) (see, *e.g.*, Wetzel et al. (1990) Deficiency of the murine fifth complement component (C5): a 2-base pair deletion in a 5' exon, *J Biol Chem* 265:2435-2440). C5 knockout mice can also be generated following standard procedures known in the art.

Complement C3 Gene and Protein

[000117] The C3 gene encodes the serum complement protein C3, which plays a central role in the activation of the classical and alternative complement activation pathways.

[000118] The human C3 gene is located on chromosome 19 at 19p13.3-p13.2. The human C3 gene has 41 exons and encodes a precursor polypeptide of 1663 amino acids, including a 22 amino acid signal peptide, a 645 amino acid β chain and a 992 amino acid α chain. During complement activation the α chain is cleaved, thereby generating 9 different peptides, including a 77 amino acid C5a, which is a potent pro-inflammatory anaphylatoxin.

[000119] C3 deficiency in humans is associated with increased susceptibility

to bacterial infections.

[000120] The C3 gene is conserved between several species, including primates, *e.g.*, chimpanzee, Rhesus monkey, other mammals, *e.g.*, dog, cow, rodent, *e.g.*, mouse, chicken, zebrafish and frog.

[000121] The mouse C3 gene is located on chromosome 17 at 17 29.72 cM19. The mouse C3 gene has 41 exons and encodes a precursor polypeptide of 1663 amino acids, including a 24 amino acid signal peptide, a 642 amino acid β chain and a 993 amino acid α chain. During complement activation the α chain is cleaved, thereby generating 9 different peptides, including a 78 amino acid C3a, which is a potent pro-inflammatory anaphylatoxin.

[000122] C3 knockout mice have been generated following standard procedures known in the art (see, *e.g.*, Drouin et al. (2001) Cutting edge: the absence of 3 demonstrates a role for complement in Th2 effector functions in a murine model of pulmonary allergy, *J Immunology* 167:4141-4144).

Species Specificity of C5 and C3 Complement Proteins

[000123] Shown herein, humanized C5 mice are functionally C5 deficient because at least some of the endogenous complement proteins display species-specificity, *i.e.*, the endogenous mouse complement proteins are unable to functionally interact with the human C5 protein. Serum obtained from humanized C5 mice or C5 knockout mice were tested for complement activity; no complement hemolytic activity was observed unless both human C3 and human C5 proteins were added.

[000124] Also shown herein, addition of human C3 to sera obtained from C3 knockout mice was able to rescue hemolytic function; complement activity was observed in C3 knockout mice when human C3 protein was added to serum.

[000125] The complement activity of C5 protein is species-specific, *i.e.*, human C5 protein is unable to substitute for mouse C5 protein in a mouse serum complement activity (*i.e.*, hemolysis) assay.

[000126] The complement activity of C3 protein, in contrast does not appear

to be similarly species-specific, *i.e.*, human C3 protein is able to substitute for mouse C3 protein in a mouse serum complement activity (*i.e.*, hemolysis) assay.

Therapeutic Inhibition of the Complement System

[000127] Two targets for therapeutic complement inhibition are the complement activation peptides C5a and C3a, which are generated upon complement activation by enzymatic cleavage in both the classical and alternative pathways from complement proteins C5 and C3, respectively (*see* Markides (1998); Mollnes et al. (2006)).

[000128] For example, blocking C5 cleavage by antibodies, for example, antibodies disclosed in US Pat. No. 6,355,245, is a possible therapeutic strategy for complement inhibition in diseases associated with complement activation.

Species Specificity of Human C5 and C3 Inhibitors

[000129] Candidate therapeutic molecules that target the complement proteins C5 or C3 are typically evaluated for pharmacokinetics (PK) and pharmacodynamics (PK) in non-human animals, *e.g.*, rodents, *e.g.*, mice or rats. Such therapeutic molecules are also tested for *in vivo* therapeutic efficacy in non-human animal, *e.g.*, rodent, *e.g.*, mouse or rat, models of human diseases, disorders and conditions associated with complement activation.

[000130] However, therapeutic molecules, which are specific for the human complement proteins C5 or C3, *i.e.*, human-specific C5 or C3 inhibitors, cannot be adequately evaluated for PD or *in vivo* therapeutic efficacy in rodents, in particular mice, because the targets of these therapeutic molecules are missing. This problem is not overcome using transgenic non-human animals, *e.g.*, rodents, *e.g.*, mice or rats, expressing human C5 or C3 complement proteins because of the above-mentioned species specificity of these proteins.

[000131] Accordingly, to assess the PD and *in vivo* therapeutic efficacy of a human-specific C5 or C3 protein antagonist or inhibitor in non-human animals, *e.g.*, rodents, *e.g.*, mice or rats, it is desirable to replace the endogenous C5 and/or C3 proteins with human C5 and/or C3 proteins. Moreover, in order to

avoid potential problems of over- or under-expression of the human C5 and/or C3 proteins, it is desirable to insert the human C5 and/or C3 genes into the genome of the non-human animals, *e.g.*, rodents, *e.g.*, mice or rats, at the endogenous C5 and/or C3 gene loci, and to express the human C5 and/or C3 proteins in non-human animals, *e.g.*, rodents, *e.g.*, mice or rats, under the control, at least in part, of the endogenous C5 and/or C3 regulatory elements.

Creation of Humanized C3 and/or C5 Non-human Animals

[000132] Methods for generating the humanized C3 and/or C5 animals of the present invention are well known in the art (*see*, generally, Gene Targeting: A Practical Approach, Joyner, ed., Oxford University Press, Inc. (2000)). In one embodiment, generation of the mice may optionally involve disruption of murine C3 and/or C5 genes and introduction of the gene encoding human or humanized C3 and/or C5 into the murine genome. In one embodiment, the introduction of the gene encoding human or humanized C3 and/or C5 is at the same location as the endogenous murine C3 and/or C5 genes.

[000133] The transgenic non-human animals of the invention can be produced by introducing transgenes into the germline of the animal. Embryonic target cells at various developmental stages can be used to introduce transgenes. Different methods are used depending on the stage of development of the embryonic target cell. The specific line(s) of any animal used to practice this invention are selected for general good health, good embryo yields, good pronuclear visibility in the embryo, and good reproductive fitness. When transgenic mice are to be produced, strains such as C57BL/6 or C57BL/6 x DBA/2 F_1, or FVB lines are often used (obtained commercially from Charles River Labs, Boston, Mass., The Jackson Laboratory, Bar Harbor, ME, or Taconic Labs.).

[000134] Introduction of the transgene into the embryo can be accomplished by any means known in the art such as, for example, microinjection, electroporation, or lipofection. For example, the transgene(s) can be introduced

into a mammal by microinjection of the construct into the pronuclei of the fertilized mammalian egg(s) to cause one or more copies of the construct to be retained in the cells of the developing mammal(s). Following introduction of the transgene construct into the fertilized egg, the egg may be incubated *in vitro* for varying amounts of time, or reimplanted into the surrogate host, or both. *In vitro* incubation to maturity is within the scope of this invention. One common method is to incubate the embryos *in vitro* for about 1-7 days, depending on the species, and then reimplant them into the surrogate host.

[000135] Reimplantation is accomplished using standard methods. Usually, the surrogate host is anesthetized, and the embryos are inserted into the oviduct. The number of embryos implanted into a particular host will vary by species, but will usually be comparable to the number of off spring the species naturally produces.

[000136] Retroviral infection can also be used to introduce transgene into a non-human animal. The developing non-human embryo can be cultured *in vitro* to the blastocyst stage. During this time, the blastomeres can be targets for retroviral infection (Jaenich, R. (1976) *PNAS* 73:1260-1264). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona pellucida (*Manipulating the Mouse Embryo*, Hogan eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1986). The viral vector system used to introduce the transgene is typically a replication-defective retrovirus carrying the transgene (Jahner et al. (1985) *PNAS* 82:6927-6931; Van der Putten et al. (1985) *PNAS* 82:6148-6152).

[000137] A third type of target cell for transgene introduction is the embryonic stem (ES) cell. Transgenes can be efficiently introduced into the ES cells by DNA transfection or by retrovirus-mediated transduction. Such transformed ES cells can thereafter be combined with blastocysts from a non-human animal. The ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal.

[000138] Transgenic animals comprising humanized C3 and/or C5 can be crossed with other animals. A manner of preparation is to generate a series of mammals, each containing one of the desired constructs or transgenes. Such mammals are bred together through a series of crosses, backcrosses and selections, to ultimately generate a single mammal containing all desired constructs and/or transgenes, where the mammal is otherwise congenic (genetically identical) to the wild type except for the presence of the desired constructs and/or transgene(s). In one embodiment, a mouse comprising a human or humanized C3 and/or C5 gene is produced in this manner.

[000139] Typically, crossing and backcrossing is accomplished by mating siblings or a parental strain with an offspring, depending on the goal of each particular step in the breeding process. In certain cases, it may be necessary to generate a large number of offspring in order to generate a single offspring that contains each of the knockout constructs and/or transgenes in the proper chromosomal location. In addition, it may be necessary to cross or backcross over several generations to ultimately obtain the desired genotype.

Use of Humanized C3 and/or C5 Non-human Animals for Identifying Compounds Capable of Modulating the Complement System

[000140] In some aspects, provided herein are methods for identifying a candidate therapeutic molecule (*i.e.* a compound) capable of modulating complement activation. The method utilizes any of the humanized C3 and/or C5 rodents (for example, mice or rats) disclosed herein. In some embodiments, candidate compounds are administered directly to the rodents following experimental induction of complement activation (for example, in a kidney ischemia/reperfusion model) and the effects of said compounds with respect to their ability to modulate the complement system are assessed. In other embodiments, candidate compounds are contacted with serum obtained from these animals and complement activity is assessed using any commonly used *in vitro* assessment technique (such as, but not limited to CH_{50} assays).

[000141] In some embodiments, the candidate compound can modulate complement activation by reducing, decreasing, or inhibiting complement activation. The compound may reduce complement activation in any of the rodents disclosed herein by any of 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% in comparison to control rodents that are not treated with the candidate compound.

[000142] In another embodiment, the candidate compound can reduce, decrease, or inhibit complement activation for up to 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10, hours, 11, hours, 12 hours, 18 hours, 24 hours, 30 hours, 36 hours, 42 hours, 48 hours, 54 hours, 60 hours, 66 hours, 72 hours, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, or three weeks, inclusive (including any periods of time in between these values).

[000143] The candidate compound can further modulate complement activation in other embodiments by increasing, amplifying or activating complement activity. The compound may increase complement activation in any of the rodents disclosed herein by any of 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, 99%, or 100% in comparison to control rodents that are not treated with the candidate compound.

[000144] In another embodiment, the candidate compound can increase, amplify or activate complement activity for up to 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10, hours, 11, hours, 12 hours, 18 hours, 24 hours, 30 hours, 36 hours, 42 hours, 48 hours, 54 hours, 60 hours, 66 hours, 72 hours, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, or three weeks, inclusive (including any periods of time in between these values).

[000145] Candidate compounds can be, without limitation, small molecule chemical compounds, antibodies, proteins, inhibitory nucleic acids, or any combination thereof.

1. Antibodies

[000146] In some aspects, the candidate compound binds (such as preferentially binds) to a complement protein (such as, but not limited to, C5 or C3 (e.g. C3a)) and is an antibody. In some embodiments, the antibodies are C5 and/or C3 antagonists and can decrease complement activation. In other embodiments, the antibodies are C5 and/or C3 agonists and can increase complement activation.

[000147] Variants of antibodies can also be made based on information known in the art, without substantially affecting the activity of antibody. For example, antibody variants can have at least one amino acid residue in the antibody molecule replaced by a different residue. For antibodies, the sites of greatest interest for substitutional mutagenesis generally include the hypervariable regions, but framework region (FR) alterations are also contemplated.

[000148] For antibodies, one type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (*e.g.* 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibodies thus generated are displayed from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.* binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding.

[000149] Nucleic acid molecules encoding amino acid sequence variants of the antibody can be prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody.

[000150] It may be desirable to introduce one or more amino acid modifications in an Fc region of the immunoglobulin polypeptides of the invention, thereby generating a Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.* , a human IgGl, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions including that of a hinge cysteine.

[000151] Fc region variants with altered (*i.e.* improved or diminished) Clq binding and/or Complement Dependent Cytotoxicity (CDC) are described in

International Patent Application Publication No.: W099/51642 (incorporated herein by reference). Such variants may comprise an amino acid substitution at one or more of amino acid positions of the Fc region. See, also, Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and International Patent Application Publication No.:W094/29351 concerning Fc region variants, the disclosures of each of which are incorporated by reference herein.

2. Non-antibody binding polypeptides

[000152] In some aspects, the candidate compound binds (such as preferentially binds) to a complement protein (such as, C5 or C3 (*e.g.* C3a)) and is a non-antibody binding polypeptide. In some embodiments, the non-antibody binding polypeptide is a C5 and/or C3 antagonist and can decrease complement activation. In other embodiments, the non-antibody binding polypeptide is a C5 and/or C3 agonist and can increase complement activation.

[000153] Binding polypeptides may be chemically synthesized using known polypeptide synthesis methodology or may be prepared and purified using recombinant technology. Binding polypeptides are usually at least about 5 amino acids in length, alternatively at least about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 amino acids in length or more, wherein such binding polypeptides that are capable of binding to a target, such as any of the complement proteins (*e.g.* C5 or C3) discussed herein.

[000154] Binding polypeptides may be identified without undue experimentation using well known techniques. In this regard, it is noted that techniques for screening polypeptide libraries for binding polypeptides that are

capable of binding to a polypeptide target are well known in the art (*see, e.g.*, U.S. Patent Nos. 5,556,762, 5,750,373, 4,708,871, 4,833,092, 5,223,409, 5,403,484, 5,571,689, 5,663,143; PCT Application Publication Nos. WO 84/03506 and WO84/03564; Geysen et al, *Proc. Natl. Acad. Sci. U.S.A.*, 81:3998-4002 (1984); Geysen et al, *Proc. Natl. Acad. Sci. U.S.A.*, 82: 178-182 (1985); Geysen et al., *J. Immunol. Meth*,, 102:259-274 (1987); Clackson, T. et al., (1991) Nature, 352: 624; Kang, A.S. et al., (1991) Proc. Natl. Acad. Sci. USA, 88:8363, and Smith, G. P. (1991) Current Opin. Biotechnol, 2:668, the disclosures of each of which are incorporated by reference herein.

[000155] Methods for generating peptide libraries and screening these libraries are also disclosed in U.S. Patent Nos. 5,723,286, 5,432,018, 5,580,717, 5,427,908, 5,498,530, 5,770,434, 5,734,018, 5,698,426, 5,763,192, and 5,723,323, the disclosures of each of which are incorporated by reference herein.

[000156] Binding polypeptides can be modified to enhance their inhibitory and/or therapeutic effect (including, for example, enhanced affinity, improved pharmacokinetic properties such as half-life, stability, and clearance rate, reduced toxicity, etc.). Such modifications include, without limitation, glycosylation, pegylation, substitution with non-naturally occurring but functionally equivalent amino acid, linking groups, etc.

3. Small molecules

[000157] In some aspects, the candidate compound binds (such as preferentially binds) to a complement protein (such as, C5 or C3 (*e.g.* C3a)) and is a small molecule. In some embodiments, the small molecule is a C5 and/or C3 antagonist and can decrease complement activation. In other embodiments, the small molecule is a C5 and/or C3 agonist and can increase complement activation.

[000158] Small molecules are preferably organic molecules other than binding polypeptides or antibodies as defined herein. Organic small molecules may be identified and chemically synthesized using known methodology (see, e.g., PCT Application Publication Nos. WO 00/00823 and WO 00/39585). Organic small molecules are usually less than about 2000 Daltons in size, alternatively less than about 1500, 750, 500, 250 or 200 Daltons in size, wherein such organic small molecules that are capable of binding to a polypeptide as described herein may be identified without undue experimentation using well known techniques. In this regard, it is noted that techniques for screening organic small molecule libraries for molecules that are capable of binding to a polypeptide target are well known in the art (see, e.g., PCT Application Publication Nos. WO 00/00823 and WO 00/39585).

[000159] Organic small molecules may be, for example, aldehydes, ketones, oximes, hydrazones, semicarbazones, carbazides, primary amines, secondary amines, tertiary amines, N- substituted hydrazines, hydrazides, alcohols, ethers, thiols, thioethers, disulfides, carboxylic acids, esters, amides, ureas, carbamates, carbonates, ketals, thioketals, acetals, thioacetals, aryl halides, aryl sulfonates, alkyl halides, alkyl sulfonates, aromatic compounds, heterocyclic compounds, anilines, alkenes, alkynes, diols, amino alcohols, oxazolidines, oxazolines, thiazolidines, thiazolines, enamines, sulfonamides, epoxides, aziridines, isocyanates, sulfonyl chlorides, diazo compounds, acid chlorides, or the like.

[000160] In some aspects, the small molecule chemical compound is a component of a combinatorial chemical library. Combinatorial chemical libraries are a collection of multiple species of chemical compounds comprised of smaller subunits or monomers. Combinatorial libraries come in a variety of sizes, ranging from a few hundred to many hundreds of thousand different species of chemical compounds. There are also a variety of library types, including oligomeric and polymeric libraries comprised of compounds such as carbohydrates, oligonucleotides, and small organic molecules, etc. Such libraries have a variety

of uses, such as immobilization and chromatographic separation of chemical compounds, as well as uses for identifying and characterizing ligands capable of binding a target molecule (for example, C5 and/or C3) or mediating a biological activity of interest (such as, but not limited to, inhibition or activation of complement activity).

[000161] Various techniques for synthesizing libraries of compounds on solid-phase supports are known in the art. Solid-phase supports are typically polymeric objects with surfaces that are functionalized to bind with subunits or monomers to form the compounds of the library. Synthesis of one library typically involves a large number of solid-phase supports. To make a combinatorial library, solid-phase supports are reacted with one or more subunits of the compounds and with one or more numbers of reagents in a carefully controlled, predetermined sequence of chemical reactions. In other words, the library subunits are "grown" on the solid-phase supports. The larger the library, the greater the number of reactions required, complicating the task of keeping track of the chemical composition of the multiple species of compounds that make up the library. In some embodiments, the small molecules are less than about 2000 Daltons in size, alternatively less than about 1500, 750, 500, 250 or 200 Daltons in size.

[000162] The small molecule agents described in any of the aspects herein can be derived from any type of chemical reaction that can be carried out on a solid support. Such chemical reactions include, but are not limited to, 2+2 cycloadditions including trapping of butadiene; [2+3] cycloadditions including synthesis of isoxazolines, furans and modified peptides; acetal formation including immobilization of diols, aldehydes and ketones; aldol condensation including derivatization of aldehydes, synthesis of propanediols; benzoin condensation including derivatization of aldehydes; cyclocondensations including benzodiazepines and hydantoins, thiazolidines, turn mimetics, porphyrins, phthalocyanines; Dieckmann cyclization including cyclization of diesters; Diels-

Alder reaction including derivatization of acrylic acid; Electrophilic addition including addition of alcohols to alkenes; Grignard reaction including derivatization of aldehydes; Heck reaction including synthesis of disubstituted alkenes; Henry reaction including synthesis of nitrile oxides *in situ* (see 2+3 cycloaddition); catalytic hydrogenation including synthesis of pheromones and peptides (hydrogenation of alkenes); Michael reaction including synthesis of sulfanyl ketones, bicyclo[2.2.2]octanes; Mitsunobu reaction including synthesis of aryl ethers, peptidyl phosphonates and thioethers; nucleophilic aromatic substitutions including synthesis of quinolones; oxidation including synthesis of aldehydes and ketones; Pausen-Khand cycloaddition including cyclization of norbornadiene with pentynol; photochemical cyclization including synthesis of helicenes; reactions with organo-metallic compounds including derivatization of aldehydes and acyl chlorides; reduction with complex hydrides and tin compounds including reduction of carbonyl, carboxylic acids, esters and nitro groups; Soai reaction including reduction of carboxyl groups; Stille reactions including synthesis of biphenyl derivatives; Stork reaction including synthesis of substituted cyclohexanones; reductive amination including synthesis of quinolones; Suzuki reaction including synthesis of phenylacetic acid derivatives; and Wittig-Horner reactions including reactions of aldehydes, pheromones, and sulfanyl ketones.

[000163] References disclosing the synthesis of chemical libraries as well as the deconvolution of the individual compounds of those libraries onto individual solid phase supports, can be found in U.S. Patent Application No. 2009/0032592; Needels et al., (1993), *Proc. Natl. Acad. Sci. USA* 90: 10700-10704; and PCT Application Publication No. WO 97/15390, the disclosures of which are incorporated by reference herein.

4. Inhibitory nucleic acids

[000164] In one aspect of this invention, the candidate complement modulatory compound is one or more oligonucleotides targeted to a component

(such as an mRNA) of the complement system. The inhibitory nucleic acid can be, without limitation, any of an antisense oligonucleotide, a small inhibitory RNA (siRNA), or a ribozyme.

[000165] The oligonucleotide of the invention may be an mRNA encoding a protein component of the complement system. The oligonucleotides will bind to the mRNAs and interfere with their functions, either by mediating their destruction or by preventing translation into proteins. Absolute complementarity, although preferred, is not required. An oligonucleotide sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex. The ability to hybridize will depend on both the degree of complementarity and the length of the oligonucleotide. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex. Those skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

[000166] In general, complementary oligonucleotides manufactured to hybridize with mRNAs for proteins of the complement system are targeted to any region of the mRNA, including to the 5′ untranslated region of the mRNA, to the complement of the AUG start codon, or to the 3′ untranslated region.

[000167] The oligonucleotides can have alternate internucleoside linkages, such as, but not limited to, phosphorothioate (Mag at al., *Nucleic Acids Res.* 19:1437-1441, 1991; and U.S. Pat. No. 5,644,048), peptide nucleic acid or PNA (Egholm, *Nature*, 3685:566-568, 1993; and U.S. Pat. No. 6,656,687), phosphoramide (Beaucage, *Methods Mol. Biol.* 20:33-61, 1993), phosphorodithioate (Capaldi et al., *Nucleic Acids Res.*, 28:E40, 2000). Other oligonucleotide analogs include such as, but not limited to, morpholino (Summerton, *Biochim. Biophys. Acta*, 1489:141-158, 1999), locked oligonucleotides (Wahlestedt wt al., *Proc. Natl. Acad. Sci. USA*, 97:5633-5638,

2000), peptidic nucleic adds or PNA (Nielsen et al., 1993; Hyrup and Nielsen, 1996) or 2-o-(2-methoxy)ethyl modified 5′ and 3′ end oligonucleotides (McKay et al., *J. Biol. Chem.*, 274:1715-1722, 1999). All of these references are hereby expressly incorporated by reference. The nucleic acids may contain any combination of deoxyribo- and ribo-nucleotides, and any combination of bases, including uracil, adenine, thymine, cytosine, guanine, inosine, xathanine hypoxathanine, isocytosine, isoguanine, etc.

[000168] The complementary oligonucleotides according to the invention may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl)uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine. N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5′-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N2-carboxypropyl)uracil, $(acp3)_w$, and 2,6-diaminopurine

[000169] The complementary oligonucleotides may also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

[000170] The complementary oligonucleotides should be at least ten nucleotides in length, and may range from 10 to about 50 nucleotides in length, such as 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29,

30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides in length.

Assessing Efficacy of Candidate Compounds

[000171] Therapeutic efficacy of candidate complement modulatory compounds, appropriate dosages, and methods of the present invention in a given non-human animal (such as a rodent, *e.g.*, a mouse or a rat), can be determined in accordance with *in vitro* complement assays well known to those having ordinary skill in the art. The complement pathway generates numerous specific products during the normal course of activation and sensitive and specific assays have been developed and are available commercially for most of these activation products, including the small activation fragments C3a, C4a, and C5a and the large activation fragments Bb and sC5b-9. Most of these assays utilize monoclonal antibodies that react with new antigens exposed on the complement fragment, but not on the native proteins from which they are formed, making these assays very simple and specific. ELISA technology is particularly common, although radioimmunoassay (RIA) is also used for detection of C3a and C5a. These latter assays measure both the unprocessed fragments and their processed fragments (*i.e.*, the major forms found in the circulation). Measurement of C3a provides a sensitive, pathway-independent indicator of complement activation. Detection of the fluid-phase product of membrane attack pathway activation, sC5b-9, provides evidence that complement is being activated to completion. Further, the classical pathway also generates C4a, measurement of which provides information about the activity of C3b inhibitor and whether such an inhibitor is activating the classical pathway.

[000172] In addition, several *in vivo* models of complement activation are also available to the skilled artisan for assessing a candidate compound's ability to modulate the complement system. For example, the complement system is implicated in ischemia-reperfusion injury which is sustained after an ischemic event and subsequent restoration of blood flow. Examples of injuries that can

cause ischemia-reperfusion injury include, without limitation, myocardial infarction, cerebral ischemic events, intestinal ischemia, and many aspects of vascular surgery, cardiac surgery, trauma, and transplantation. Activation of the complement system plays a role in the inflammatory events of ischemia-reperfusion injury. The ischemia injury results in alterations of the cell membrane, affecting lipids, carbohydrates, or proteins of the external surface such that these exposed epitopes are altered and can act as neo-antigens (modified self antigens). The involvement of the classical pathway of complement to ischemia-reperfusion injury is evidenced by mice genetically deficient in either C3 or C4 that display equal protection from local injury in a hindlimb and animal model of injury (Austen et al. (2003) *Int J Immunopath* Pharm 16:1). Additionally, complement activation has also been shown be involved in a kidney model of ischemia injury (Guo et al. (2005) *Ann Rev Immunol* 23:821).

[000173] The invention can be further understood by reference to the following examples, which are provided by way of illustration and are not meant to be limiting.

EXAMPLES

Example 1

Replacement of the Endogenous Mouse C5 Gene with a Human C5 Gene

[000174] The 97 kb human C5 gene containing coding exons 2 through 42 of the human C5 gene replaced 75.8 kb of the murine C5 gene locus spanning coding exons 2 through 41 and including a portion of the 3' untranslated region. See Figure 1.

[000175] A targeting construct for replacing the mouse with the human C5 gene in a single targeting step was constructed using VelociGene® genetic engineering technology (see Valenzuela et al. (2003) High-throughput engineering of the mouse genome coupled with high-resolution expression analysis, Nature Biotech, 21(6):652-659). Mouse and human C5 DNA were obtained from bacterial artificial chromosome (BAC) clones bMQ-2277L16 and

CTD-2559I19, respectively. Briefly, a linearized targeting construct generated by gap repair cloning containing mouse C5 upstream and downstream homology arms flanking a 97 kb human C5 sequence extending from intron 1 just upstream of coding exon 2 through coding exon 41 and the 3' untranslated region (genomic coordinates: GRCh38: chr9:120,952,335-121,050,276 (- strand)) and a floxed neo selection cassette, was electroporated into F1H4 mouse embryonic stem (ES) cells (C57BL/6 x 129 F1 hybrid). Correctly targeted ES cells (MAID 7140) were further electroporated with a transient Cre-expressing vector to remove the drug selection cassette. Targeted ES cell clones without drug cassette (MAID 7141) were introduced into an 8-cell stage SW mouse embryo by the VelociMouse® method (see, U.S. Pat. Nos. 7,294,754, 7,576,259, 7,659,442, and Poueymirou et al. (2007) F0 generation mice that are essentially fully derived from the donor gene-targeted ES cells allowing immediate phenotypic analyses Nature Biotech. 25(1):91-99). VelociMice® (F0 mice fully derived from the donor ES cell) bearing the humanized C5 gene were identified by genotyping for loss of mouse allele and gain of human allele using a modification of allele assay (*see*, Valenzuela et al. (2003)).

[000176] Correctly targeted ES cell clones were identified by a loss-of-native-allele (LONA) assay (Valenzuela et al. 2003) in which the number of copies of the native, unmodified C5 gene were determined by two TaqMan™ quantitative polymerase chain reactions (qPCRs) specific for sequences in the mouse C5 gene that were targeted for deletion. The qPCR assays comprised the following primer-probe sets (written 5' to 3'): upstream forward primer, CCTCCGGTTA ACTGGTTTGT GAT (SEQ ID NO:1); upstream reverse primer, GCAGTGAATG GTAGACTTCC CA (SEQ ID NO:2); upstream probe, FAM-AGTGACTTTA CTTTGGTTGT TCTGCTCACA-BHQ (SEQ ID NO:3); downstream forward primer, CCGGGAAAGG AAACCAAGAC (SEQ ID NO:4); downstream reverse primer, CAGCACAGAC TGAGGATCCA A (SEQ ID NO:5); downstream probe, FAM-AGACTGTCAT TCTGGCCCGG ACCT-BHQ (SEQ ID NO:6); in which FAM refers to the 5-carboxyfluorescein fluorescent probe and BHQ refers to the

fluorescence quencher of the black hole quencher type (Biosearch Technologies). DNA purified from ES cell clones that have taken up the targeting vector and incorporated in their genomes was combined with TaqMan™ Gene Expression Master Mix (Life Technologies) according to the manufacturer's suggestions in a 384-well PCR plate (MicroAmp™ Optical 384-Well Reaction Plate, Life Technologies) and cycled in an Applied Biosystems Prism 7900HT, which collects fluorescence data during the course of the PCRs and determines a threshold cycle (Ct), the fractional PCR cycle at which the accumulated fluorescence reaches a pre-set threshold. The upstream and downstream C5-specific qPCRs and two qPCRs for non-targeted reference genes were run for each DNA sample. The differences in the Ct values (ΔCt) between each C5-specific qPCR and each reference gene qPCR were calculated, and then the difference between each ΔCt and the median ΔCt for all samples assayed was calculated to obtain ΔΔCt values for each sample. T he copy number of the C5 gene in each sample was calculated from the following formula: copy number = 2 x $2^{-\Delta\Delta Ct}$. A correctly targeted clone, having lost one of its native copies, will have a C5 gene copy number equal to one. Confirmation that the human C5 gene sequence replaced the deleted mouse C5 gene sequence in the humanized allele was confirmed by a TaqMan™ qPCR assay that comprises the following primer-probe sets (written 5' to 3'): human upstream forward primer, TGCTCTTAAA GGACCATCAT CAC (SEQ ID NO:7); human upstream reverse primer, GTAAACGGAT AACGGAAAGG ATAGA (SEQ ID NO:8); human upstream probe, FAM-CCATTTCCAA ATGCACTTCC CAGC-BHQ (SEQ ID NO:9); human downstream forward primer, CCCACTGAAC TATCACAGGA AACA (SEQ ID NO:10); human downstream reverse primer, GGAGATGCAT TCCAGTCTCT GTTA (SEQ ID NO:11); human downstream probe, FAM-TGAAGATGAC CTCCGGATGT AACGG-BHQ (SEQ ID NO:12).

[000177] The same LONA assay is used to assay DNA purified from tail biopsies for mice derived from the targeted ES cells to determine their C5 genotypes and confirm that the humanized C5 allele has transmitted through the

germ-line. Two pups heterozygous for the replacement are bred to generate a mouse that is homozygous for the replacement of the endogenous mouse C5 gene by the human C5 gene. Pups that are homozygous for the replacement are used for phenotyping.

[000178] The upstream junction of the murine locus and the sequence containing the human C5 gene is designed to be within 5'-TGAAAAACCT CCATTACTAC TTCTACAGAT GCCCACAGTG GTCTTGCATT CTATCCTTGT /(GCGATCGC)/ TCTGCCATCT CCTACTAGGC ATGTGGGGAA GGGGAATTCA GATGATGGTT GGAAATCTGG AAATTCTTTC CTCTCTTTTG TAATTTGCCT (SEQ ID NO:13), wherein the final mouse C5 nucleotide prior to the first nucleotide of the human C5 gene is the last T in CTTGT, and the first nucleotide of the human C5 sequence is the first T in TCTGC, which span the AsiSI site (within parentheses), wherein the precise junctions are indicated by forward slashes. The downstream junction of the sequence containing the human C5 gene and the floxed neo selection cassette is designed to be within 5'-GCAAATAGGG CAGGCCACAG TGGCCTAATT AACCCACAAT GCAGCTGTCA AATATCAAAG AAGGTTTTGT GAATTAGCCT/CTCGAGATAA CTTCGTATAA TGTATGCTAT ACGAAGTTAT ATGCATGGCC TCCGCGCCGG GTTTTGGCGC CTCCCGCGGG (SEQ ID NO:14), wherein the final nucleotide of the human C5 sequence is the T in AGCCT, and the first nucleotide of the floxed neo selection cassette is the first C in CTCGA, the precise junction being indicated by a forward slash. The downstream junction of the sequence of the murine C5 locus is designed to be within 5'-ATGTCTGGAA TAACTTCGTA TAATGTATGC TATACGAAGT TATGCTAGTA ACTATAACGG TCCTAAGGTA GCGAGCTAGC/CAGGCTATTA GGCTTCTGTC CCCAACTGTG GTGGCAAATA GGCCAGACCA CAGTCACCAG ATGAAGCCAT AAATGCAGCT (SEQ ID NO:15), wherein the final nucleotide of the floxed neo selection cassette is the second C in CTAGC, and the first nucleotide of the murine C5 locus is the first C in CAGGC, the precise junction being indicated by a forward slash. The downstream junction of the sequence containing the

human C5 gene and the murine locus is designed to be within 5'-GCAAATAGGG CAGGCCACAG TGGCCTAATT AACCCACAAT GCAGCTGTCA AATATCAAAG AAGGTTTTGT GAATTAGCCT/CTCGAG(ATAA CTTCGTATAA TGTATGCTAT ACGAAGTTAT) GCTAGTAACT ATAACGGTCC TAAGGTAGCG AGCTAGC/CA GGCTATTAGG CTTCTGTCCC CAACTGTGGT GGCAAATAGG CCAGACCACA GTCACCAGAT GAAGCCATAA ATGCAGCTGA (SEQ ID NO:16), wherein the final nucleotide of the human C5 sequence is with the "T" in AGCCT, and the first nucleotide of the mouse C5 sequence is the first "C" in CAGGC; which span a loxP site (within parentheses) that remains after removal of the floxed neo selection cassette, wherein the precise junctions are indicated by forward slashes.

Example 2

Replacement of the Endogenous Mouse C3 Gene with a Human C3 Gene

Version 1 – Replacement with Human C3 Promoter and Coding Exons 1 through 41

[000179] The human C3 gene containing 5' regulatory elements and all of the coding exons 1 through 41 of the human C3 gene replaced the murine C5 gene locus spanning 5' regulatory elements and all of the coding exons 2 through 41. The methods described above in Example 1 were basically used to replace the mouse C3 gene sequences with human C3 gene sequences. See Figure 2A.

[000180] Preliminarily, a targeted deletion of 25 kb of the mouse C3 gene was generated in mouse ES cells by replacement of coding exons 2 through 41 and including 900 bp 3' to the polyadenylation site with a floxed neo cassette. The resultant mouse ES cells, 12132 ES cells, are a heterozygous C3 knockout. 12132 cells were used to generate C3 knockout mice according to procedures known in the art.

[000181] A targeting construct was generated containing mouse C3 upstream and downstream homology arms flanking a human C3 sequence extending from 5' regulatory elements upstream of coding exon 1 through coding

exon 41 and the 3' untranslated region and a floxed hygro selection cassette, and electroporated into 12132 ES cells. Correctly targeted ES cells (MAID 6148) were further electroporated with a transient Cre-expressing vector to remove the drug selection cassette. Targeted ES cell clones without drug cassette (MAID 6149) were introduced into an 8-cell stage mouse embryo. F0 mice fully derived from the donor ES cell bearing the humanized C3 gene were identified by genotyping for loss of mouse allele and gain of human allele as described in Example 1.

[000182] Confirmation that the floxed neo cassette replaced the deleted mouse C3 gene sequence in the C3 knockout 12132 ES cells was confirmed by a TaqMan™ qPCR assay that comprises the following primer-probe sets (written 5' to 3'): 12132TU, mouse C3 intron 1: forward primer, GGCCTGATTA CATGGACCTG TC (SEQ ID NO:17); reverse primer, CCCAGGCTTG GCTGGAATG (SEQ ID NO:18); probe, FAM-TGTCCACTCT GGAAGCCCAG GC-BHQ (SEQ ID NO:19); 12132TD, 3' of mouse C3 exon 41: forward primer, GCCAGGAGAG GAAGCTGGAG (SEQ ID NO:20); reverse primer, TGGCTCAGCA GTAAAGAACA C (SEQ ID NO:21); probe, FAM-ACAGATTGCT GTGAGCTGCC CAAA-BHQ (SEQ ID NO:22); neo cassette: forward primer, GGTGGAGAGG CTATTCGGC (SEQ ID NO:23); reverse primer, GAACACGGCG GCATCAG (SEQ ID NO:24); probe, FAM-TGGGCACAAC AGACAATCGG CTG-BHQ (SEQ ID NO:25).

[000183] Confirmation that the human C3 gene sequence replaced the deleted mouse C3 gene sequence in the humanized allele was confirmed by a TaqMan™ qPCR assay that comprises the following primer-probe sets (written 5' to 3'): mC3-1, mouse C3 promoter: forward primer, GCCAGCCTAG CCTACTTCA (SEQ ID NO:26); reverse primer, GCCACCCATC CCAGTTCT (SEQ ID NO:27); probe, FAM- CAGCCCAGGC CCTTTAGATT GCA-BHQ(SEQ ID NO:28); mC3-2, mouse C3 3' untranslated region, forward primer, TACGGTGTTA GGTTCACTAT TGGT (SEQ ID NO:29); reverse primer, GTCGCCAGCA GTCTCATACA G (SEQ ID NO:30); probe, CAL Orange-

AGTGGGCATC CCTTGCCAGG C-BHQ(SEQ ID NO:31); hygro cassette: forward primer, TGCGGCCGAT CTTAGCC (SEQ ID NO:32); reverse primer, TTGACCGATT CCTTGCGG (SEQ ID NO:33); probe, FAM-ACGAGCGGGT TCGGCCCATT C-BHQ (SEQ ID NO:34); hC3-1, human C3 promoter: forward primer, GGGCCTCCTA AGTTTGTTGA GTATC (SEQ ID NO:35); reverse primer, CAGGGCTGGT TCCCTAGAAA TC (SEQ ID NO:36); probe, FAM-TACAATAGCA GGCACAGCAC CCA-BHQ (SEQ ID NO:37); hC3-2, human C3 intron 1: forward primer, GGCTGAGAGT GGGAGTCATG (SEQ ID NO:38); reverse primer, GCACTTGCCA ATGCCATTAT C (SEQ ID NO:39); probe, FAM-CTGCTGTCCT GCCCATGTGG TTG-BHQ (SEQ ID NO:40); hC3-3, human C3 exon 41: forward primer, CGAATGCCAA GACGAAGAGA AC (SEQ ID NO:41); reverse primer, GGGCACCCAA AGACAACCAT (SEQ ID NO:42); probe, CAL Orange-CAGAAACAAT GCCAGGACCT CGGC-BHQ (SEQ ID NO:43).

[000184] The same LONA assay is used to assay DNA purified from tail biopsies for mice derived from the targeted ES cells to determine their C3 genotypes and confirm that the humanized C3 allele had transmitted through the germ-line. Two pups heterozygous for the replacement are bred to generate a mouse that is homozygous for the replacement of the endogenous mouse C3 gene by the human C3 gene. Pups that are homozygous for the replacement are used for phenotyping.

[000185] The sequences of the junction of the murine C3 locus and the sequence containing the human C3 gene, the junction of the sequence containing the human C3 gene and the floxed hygro selection cassette, and the junction of the sequence of the floxed hygro selection cassette and the murine C3 locus are determined as described above in Example 1.

Version 2 - Replacement with Human C3 Coding Exons 2 Through 41

[000186] The human C3 gene containing coding exons 2 through 41 of the human C3 gene replaced the murine C5 gene locus spanning coding exons 2 through 41. The methods described above in Example 1 were basically used to replace the mouse C3 gene sequences with human C3 gene sequences. See

Figure 2B.

[000187] Preliminarily, a targeted deletion of 25 kb of the mouse C3 gene was generated in mouse ES cells by replacement of coding exons 2 through 41 and including 900 bp 3' to the polyadenylation site with a floxed neo cassette. The resultant mouse ES cells, 12132 ES cells, are a heterozygous C3 knockout.

[000188] A targeting construct was generated containing mouse C3 upstream and downstream homology arms flanking a human C3 sequence extending from upstream of coding exon 2 through coding exon 41 and the 3' untranslated region and a floxed hygro selection cassette, and electroporated into 12132 ES cells. Correctly targeted ES cells (MAID 6155) were further electroporated with a transient Cre-expressing vector to remove the drug selection cassette. Targeted ES cell clones without drug cassette (MAID 6156) were introduced into an 8-cell stage mouse embryo. F0 mice fully derived from the donor ES cell bearing the humanized C3 gene were identified by genotyping for loss of mouse allele and gain of human allele as described in Example 1.

[000189] Confirmation that the floxed neo cassette replaced the deleted mouse C3 gene sequence in the C3 knockout 12132 ES cells, and that the human C3 gene sequence replaced the deleted mouse C3 gene sequence in the humanized allele, was confirmed by a TaqMan™ qPCR assay that comprises the same primer-probe sets (written 5' to 3') as described above in Version 1.

[000190] The same LONA assay is used to assay DNA purified from tail biopsies for mice derived from the targeted ES cells to determine their C3 genotypes and confirm that the humanized C3 allele had transmitted through the germ-line. Two pups heterozygous for the replacement are bred to generate a mouse that is homozygous for the replacement of the endogenous mouse C3 gene by the human C3 gene. Pups that are homozygous for the replacement are used for phenotyping.

[000191] The sequences of the junction of the murine C3 locus and the sequence containing the human C3 gene, the junction of the sequence containing the human C3 gene and the floxed hygro selection cassette, and the

junction of the sequence of the floxed hygro selection cassette and the murine C3 locus are determined as described above in Example 1.

Example 3

Complement Hemolytic Activity Assay in Mouse Serum

[000192] Complement has been implicated in ocular inflammatory and retinal degenerative diseases (*see* Mullins et al. (2000) Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease, *FASEB J*, 14(7):835-846). Blocking C5 cleavage by monoclonal antibody (mAb) is a possible therapeutic strategy for these disorders (see Copland et al. (2009) Systemic and local anti-C5 therapy reduces the disease severity in experimental autoimmune uveoretinitis, *Clinical and Experimental Immunology*, 159:303–314). For evaluation of therapies directed at complement, such as C5 and/or C3 antagonists, assays are necessary to evaluate the function of candidate therapeutics *in vitro* and *in vivo* in appropriate models of human diseases, disorders and conditions associated with complement activation.

Methods

[000193] Complement hemolytic activity was assessed using Classical Complement Assay CH_{50} (Gilcas et al. (2001) Classical pathway evaluation, *Current Protocols in Immunology*, Unit 13.1). Mouse blood was collected from the heart and clotted in eppendorf tubes on ice for 1 hour; serum was separated by centrifugation and stored at -70°C. Sheep erythrocytes (E) were sensitized by incubation with rabbit anti-sheep hemolysin for 20 min at 37°C. Sensitized cells (E_A) were incubated for 1 hour at 37°C with doubling dilutions of treated or control serum from mouse or human. Intact E_A cells were pelleted and supernatant was removed to measure absorbance at 541 nm, an index of hemolysis. E_A cells incubated with buffer only (0% lysis) or with ddH_2O (100% lysis) served as negative and positive controls respectively. Percentage of hemolysis and CH_{50} in each well were calculated relative to the ddH_20 control (Holt et al. (2001) Targeted deletion of the CD59 gene causes spontaneous

intravascular hemolysis and hemoglobinuria, *Blood*, 98(2):442-449). Mice, including $C5^{hu/hu}$, $C5^{-/-}$, $C3^{hu/hu}$, $C3^{-/-}$, and respective control strains, were generated with Velocigene® technology as described in Examples 1 and 2. Normal, C5-depleted, and C3-depleted human sera were obtained from Quidel Corp., and used as controls or for comparison. Complement components were added to test if the hemolysis function can be restored. Tested sera were incubated for 40 min at 4°C with complement components; human C5 (Sigma-Aldrich or Quidel Corp., 50 µg/ml), human C3 (Sigma-Aldrich or Quidel Corp., 10, 400, 1200 µg/ml) and murine C5 (Regeneron Pharmaceuticals, 50µg/ml). Anti-human C5 mAb (100 µg/ml) and anti-mouse C5 mAb (Hycult Biotech, 10 µg/ml) were used to confirm the specificity and utility of this system for future drug discovery.

Results

[000194] *Wild-type mouse sera have approximately 10 times lower complement hemolytic activity compared to normal human sera.* As shown in Figure 3, the complement hemolytic activity, as determined using the CH_{50} value, was at least 10 times lower in three lots of normal or wild-type mouse sera as compared to two lots of human sera.

[000195] *Anti-C5 monoclonal antibodies block hemolysis in a species-specific manner.* As shown in Figure 4, an anti-mouse C5 monoclonal antibody (msC5Ab) blocked complement hemolytic activity in normal mouse serum, and an anti-human C5 mAb (C5Ab) blocked hemolytic activity in normal human serum and human C5-depleted serum supplemented with human C5 protein, but not in normal mouse serum.

[000196] *Human C5 and C3 proteins display species-specific hemolytic activity.* As shown in Figure 5A, human C5 and C3 proteins were able to reconstitute complement hemolytic activity of human C5- and C3-depleted human serum, respectively.

[000197] As shown in Figure 5B, sera from $C5^{-/-}$ and $C3^{-/-}$ mice showed a lack of complement activity, and humanized $C5^{hu/hu}$ mouse serum displayed no

hemolytic activity, essentially equivalent to serum from $C5^{-/-}$ and $C3^{-/-}$ mice. The addition of mouse C5 protein to humanized $C5^{hu/hu}$ mouse serum rescued complement hemolytic activity, but addition of human C5 protein to humanized $C5^{hu/hu}$ mouse serum did not rescue complement activity.

[000198] As shown in Figure 5C, the lack of complement hemolytic activity of humanized $C5^{hu/hu}$ mouse serum appears not to be due to a lack of human C5 protein, as the amount of human C5 protein present in the serum of these mice was about one third the amount of C5 protein present in normal human serum. Figure 5D shows the variation between male and female humanized C5 mice with respect to pre-dose complement C5 levels. The range of human C5 protein present in the serum of these mice ranged from 20 to 100 µg/ml, which was sufficient to show hemolytic activity in serum. Human C5 normally added in these experiments was 50µg/ml.

[000199] *Both human C5 and C3 proteins are required to reconstitute hemolytic activity in mouse C5 knockout sera*. As shown in Figure 6, complement hemolytic activity could not be recovered by adding human C5 protein to $C5^{-/-}$ mouse serum, whereas the addition of human C3 and C5 proteins together rescued hemolysis in $C5^{-/-}$ mouse serum.

[000200] *Addition of human C3 protein rescues hemolytic activity in $C5^{hu/hu}$ mouse serum*. As shown in Figure 7, human C3 protein is sufficient to reconstitute complement hemolytic activity in serum from $C5^{hu/hu}$ mice.

[000201] Based on the above results, humanized $C5^{hu/hu}$ and/or $C3^{hu/hu}$ mice are appropriate non-human animals to evaluate the PK and PD of human–specific C5 and/or C3 antagonists. Based on the above results, doubly humanized $C5^{hu/hu}$ and $C3^{hu/hu}$ mouse serum may display complement hemolytic activity without a requirement for supplementation by human C5 and/or C3 proteins, thereby constituting an appropriate mouse strain for *in vivo* testing of the therapeutic efficacy of human-specific C5 and/or C3 antagonists.

Conclusions

[000202] Wild-type mouse serum has dramatically lower complement hemolytic activity compared to human serum. Genetically modified mice expressing human C5 are functional murine C5 knockouts, possibly because mouse convertase cannot cleave human C5 protein. Humanized C5 mouse serum needs the addition of human C3 protein to achieve wild-type levels of complement hemolytic activity.

Example 4

Uses for Humanized C5 and/or C3 Mice

[000203] Humanized C5 and/or C3 mice are useful to evaluate the pharmacodynamics (PD) of human-specific C5 and/or C3 compounds (such as, antagonists, *e.g.,* neutralizing anti-C5 or anti-C3 antibodies).

[000204] Pharmacokinetics (PK) and PD assays in humanized C5 and/or C3 mice are performed according to standard procedures known in the art, for example, as described in US Pat. No. 6,355,245 for an anti-C5 antibody.

[000205] Humanized C5 and/or C3 mice are useful to test the *in vivo* therapeutic efficacy of human-specific C5 or C3 antagonists, *e.g.*, neutralizing anti-C5 or anti-C3 antibodies, in a variety of disease models, for example and not for limitation: dry age-related macular degeneration (AMD); wet AMD; experimental autoimmune uveoretinitis (EAU); ocular hypertension; and optic nerve injury. (See, *e.g.*, Makrides (1998) and Mollnes et al. (2006) for a list of diseases, disorders and conditions associated with complement activation).

[000206] Dry AMD can be induced in humanized C5 and/or C3 mice by high fat diet and blue light damage (see, *e.g.*, *Exp Eye Res.* 2002, 75(5):543-553), cigarette smoke (see, *e.g.*, *Invest Ophthalmol Vis Sci.* 2006, 47(2):729-737), carboxyethylpyrrole (CEP) (see, *e.g.*, *Invest. Ophthalmol. Vis. Sci.* 2010, 51:1276-1281; Nat Med. 2012, 18(5):791-798).

[000207] The humanized C5 and/or C3 mice can also be bred to other transgenic AMD models, such as, for example, ApoE(-/-)(see, *e.g.*, *Invest.*

Ophthalmol. Vis. Sci. 2000, 41:2035-2042), Mdm1 (see, *e.g.*, *Hum. Mol. Genet.* 2008, 17:3929-3941), Sod1(-/-)(*see*, *e.g.*, *PNAS* 2006; 103:11282-11287), LDLR(-/-)(see, *e.g.*, *Br J Ophthalmol* 2005, 89:1627-1630), and DICER1 (-/-) (see, *e.g.*, *Nature*. 2011; 471(7338):325-330).

[000208] Wet AMD can be induced in humanized C5 and/or C3 mice by laser-induced choroidal neovascularization (CNV) (see *Nat Protoc*. 2013, 8(11):2197-2211).

[000209] EAU can be induced in humanized C5 and/or C3 mice (see, *e.g.*, *Clin Exp Immunol*. 2010, 159(3): 303–314).

[000210] Ocular hypertension can be induced in humanized C5 and/or C3 mice (*see*, *e.g.*, *Experimental Eye Research* 2006, 83:620-628).

[000211] Optic nerve injury can be induced in humanized C5 and/or C3 mice (see, *e.g.*, *J Neurotrauma* 2003, 20(9):895-904.

[000212] Humanized C5 mice can be used to evaluate human-specific C5 antagonists in a variety of disease models, for example and not for limitation: paroxysmal nocturnal hematuria; neuromyletic optica; renal transplantation; and kidney injury. (*See*, *e.g.*, US Pat. No. 6,355,245.)

[000213] Therapeutic efficacy assays using humanized C5 and/or C3 mice are performed according to standard procedures known in the art, for example, as described in US Pat. No. 6,355,245 for an anti-C5 antibody.

Example 5

Pharmacokinetic/Pharmacodynamic Study of an Anti-C5 Antibody in Humanized C5 Homozygous Mice

[000214] This example utilizes male and female humanized C5 homozygous mice prepared according to the prior Examples to test the ability of an anti-C5 antibody to decrease complement activation.

Methods

[000215] A total of 25 $C5^{hu/hu}$ mice (males and females) were used in the study. Serum (20 µL) was collected from two mice at each designated timepoint,

which included a "predose" timepoint as well as timepoints at 3h, 6h, 1d, 2d, 4d, 1W, 2W, 4W following subcutaneous administration of 15 mg/kg of an anti-human C5 monoclonal antibody. Samples were stored at -20°C until assayed for pharmacokinetics (PK) and pharmacodynamics (PD) alteration of complement activation.

[000216] Complement hemolytic activity was assessed as described above in Example 3. As above, human C3 (Sigma-Aldrich or Quidel Corp., 400 and 800 µg/ml) was added to serum to recapitulate the human complement system.

Results

[000217] As shown in Figure 8, subcutaneous injection of 15 mg/kg of an anti-human C5 antibody decreases complement activation for at least one week post injection. This effect was observed irrespective of whether 400 µg/ml (Figure 8A) or 800 µg/ml (Figure 8B) human C3 protein was added to the hemolytic assays.

[000218] As shown in Figure 9, PK analysis of C5 monoclonal antibody revealed clearance over time of this antibody with 2 out of 5 mice having undetectable levels by day 28.

Example 6

Cross Activity of Human and Mouse Complement Proteins

[000219] This Example investigated the cross-activity of human and mouse C3 and C5 proteins using the engineered mice described in the previous examples. As discussed in Example 3 and as shown in Figure 6, the human C5 and the mouse C3 proteins are not sufficient to reconstitute hemolytic activity in mice lacking endogenous C5. In this study, the ability of mouse C5 and human C3 to restore hemolytic activity in C3 knockout mice was observed.

Methods

[000220] Complement hemolytic activity was assessed as described in Example 3.

[000221] Mice, including $C5^{-/-}$, $C3^{-/-}$, and respective control strains, were

generated with Velocigene® technology as described in Examples 1 and 2. Normal, C3-depleted human sera were obtained from Quidel Corp. and used as controls or for comparison. Complement components were added to test if the hemolysis function can be restored. Tested sera were incubated for 40 min at 4°C with complement components; human C3 (Sigma-Aldrich or Quidel Corp., 50, 100, 500, 1200 µg/ml); and human C5 (Sigma-Aldrich or Quidel Corp., 50 µg/ml),). Anti-human C5 mAb (100 µg/ml) and anti-mouse C5 mAb (Hycult Biotech, 10 µg/ml) were used to confirm the specificity and utility of this system for future drug discovery.

Results

[000222] As shown in Figure 10A, addition of human C3 protein to wild type mice serum can enhance hemolytic function over that observed in wild type controls. However, when compared to the addition of human C3 protein back into C3-depleted human serum, the observed enhancement is less than that shown in humans. Further, as illustrated in Figure 10B, percentage hemoloysis is restored using humanized $C5^{hu/hu}$ mouse serum supplemented with human C3 protein.

[000223] When this experiment was repeated using sera derived from C3 knockout animals, it was shown that adding human C3 protein to $C3^{-/-}$ serum rescues hemolytic function, particularly with increasing concentrations of added human C3 (Figure 11).

[000224] In contrast, Figure 12 shows that addition of human C3 to C5 null animal-derived sera does not rescue hemolytic function.

Conclusions

[000225] In summary, the data derived from the present example demonstrates that while human C3 is able to cross-react with murine C5, mouse C3 is similarly unable to cross-react with human C5 to recapitulate complement activity *in vitro.* A summary of these results is presented below in Table 1.

Table 1: Summary of mouse and human C3, C5 cross-activity

mouse	+ human	Classical pathway complement activity
WT	C3	>WT
WT	C5	
WT	C3, C5	
C3KO	C3	=WT
C3KO	C5	
C3KO	C3, C5	=WT
C5KO	C3	0
C5KO	C5	0
C5KO	C3, C5	≥WT

CLAIMS

1. A rodent, comprising a replacement at an endogenous rodent C5 locus of a rodent gene sequence encoding an exon of a C5 gene with a nucleic acid sequence encoding at least one exon of a human C5 gene to form a modified C5 gene, wherein expression of the modified C5 gene is under control of rodent regulatory elements at the endogenous rodent C5 locus.

2. The rodent of claim 1, wherein the rodent is a mouse or a rat.

3. The rodent of claim 1 or claim 2, wherein the human C5 gene encoding human C5 protein comprises exon 2 through exon 41 of the human C5 gene.

4. The rodent of any one of claims 1-3, wherein the rodent is a mouse that is incapable of expressing a mouse C5 protein.

5. The rodent of any one of claims 1-4, wherein the rodent is a mouse that expresses a mouse C3 protein encoded by an endogenous mouse C3 gene.

6. The rodent of any one of claims 1-5, wherein the rodent is a mouse that expresses a human or humanized C3 protein.

7. The rodent of claim 6, wherein the mouse comprises a replacement at an endogenous mouse C3 locus of a mouse gene encoding C3 protein with a human C3 gene encoding a human C3 protein.

8. The rodent of claim 6 or claim 7, wherein expression of the human C3 gene is under control of mouse regulatory elements at the endogenous mouse C3 locus.

9. The rodent of any one of claims 1-9, wherein the rodent is a mouse that expresses human C5 protein in serum at a concentration of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190% or 200% of the level of mouse C5 protein present in the serum of an age-matched mouse that expresses functional endogenous mouse C5 protein, but does not comprise said replacement.

10. The rodent of any one of claims 1-10, wherein the rodent is a mouse that expresses human C5 protein in serum at a concentration of between about 10% and about 200%, between about 20% and about 150%, or between about 30% and about 100% of the level of mouse C5 protein present in the serum of an age-matched mouse that expresses functional endogenous C5 protein, but does not comprise said replacement.

11. The rodent of any one of claims 1-11, wherein the rodent is a mouse that expresses human C5 protein in serum at a concentration of between about 10 μg/ml and about 150 μg/ml, between about 10 μg/ml and about 125 μg/ml, or between about 15 μg/ml and about 100 μg/ml.

12. The rodent of any one of claims 1-12, wherein the rodent is a mouse that expresses human C5 protein in serum at a concentration of at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125 or 150 μg/ml.

13. A rodent, comprising a replacement at an endogenous rodent C3 locus of a rodent gene sequence encoding an exon of a C3 gene with a nucleic acid sequence encoding at least one exon of a human C3 gene to form a modified C3 gene, wherein expression of the modified C3 gene is under control of rodent regulatory elements at the endogenous rodent C5 locus.

14. The rodent of claim 13, wherein the rodent is a mouse or a rat

15. The rodent of claim 13 or claim 14, wherein the human C3 gene encoding human C3 protein comprises exon 1 through exon 41 of the human C3 gene.

16. The rodent of any one of claims 13-15, wherein the human C3 gene encoding human C3 protein comprises exon 2 through exon 41 of the human C3 gene.

17. The rodent of any one of claims 13-16, wherein the rodent is a mouse that is incapable of expressing a mouse C3 protein.

18. The rodent of any one of claims 13-17, wherein the rodent is a mouse that expresses a mouse C5 protein encoded by an endogenous mouse C5 gene.

19. The rodent of claim 18 or claim 19, wherein the mouse expresses a human or humanized C5 protein.

20. The rodent of claim 19, wherein the mouse comprises a replacement at an endogenous mouse C5 locus of a mouse gene encoding C5 protein with a human C5 gene encoding human C5 protein.

21. The rodent of claim 19 or claim 20, wherein expression of the human C5 gene is under control of mouse regulatory elements at the endogenous mouse C5 locus.

22. The rodent of any one of claims 13-21, wherein the rodent is a mouse that expresses human C3 protein in serum at a concentration of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%,

160%, 170%, 180%, 190% or 200% of the level of mouse C3 protein present in the serum of an age-matched mouse that expresses functional endogenous mouse C3 protein, but does not comprise said replacement.

23. The rodent of any one of claims 13-22, wherein the rodent is a mouse that expresses human C3 protein in serum at a concentration of between about 10% and about 200%, between about 20% and about 150%, or between about 30% and about 100% of the level of mouse C3 protein present in the serum of an age-matched mouse that expresses functional endogenous mouse C3 protein, but does not comprise said replacement.

24. The rodent of any one of claims 13-23, wherein the rodent is a mouse that expresses human C3 protein in serum at a concentration of between about 100 μg/ml and about 1500 μg/ml, between about 200 μg/ml and about 1250 μg/ml, or between about 300 μg/ml and about 1000 μg/ml.

25. The rodent of any one of claims 13-24, wherein the rodent is a mouse that expresses human C3 protein in serum at a concentration of at least about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1250 or 1500 μg/ml.

26. A method for making a humanized rodent, comprising replacing a rodent C5 gene sequence encoding rodent C5 protein with a human C5 gene sequence comprising one or more exons of the human C5 gene sequence encoding human or humanized C5 protein, wherein the replacement is at an endogenous rodent C5 locus and the human C5 gene sequence comprising one or more exons of the human C5 gene sequence encoding human or humanized C5 protein is operably linked to rodent regulatory elements or sequences at the endogenous rodent C5 locus.

27. The method of claim 26, wherein the rodent is a mouse or a rat.

28. The method of claim 26 or claim 27, wherein the rodent regulatory elements or sequences are from a mouse or a rat.

29. The method of any one of claims 26-28, wherein the rodent regulatory elements or sequences are endogenous rodent regulatory elements or sequences at the rodent C5 locus.

30. The method of any one of claims 26-29, wherein the human C5 gene sequence replacing the rodent C5 gene sequence comprises at least one exon of the human C5 gene sequence.

31. The method of claim 30, wherein the human C5 gene sequence replacing the rodent C5 gene sequence comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 exons of the human C5 gene sequence.

32. The method of claim 30 or claim 31, wherein the human C5 gene sequence replacing the rodent C5 gene sequence comprises all 41 exons of the human C5 gene sequence.

33. The method of any one of claims 26-32, wherein the replacement is at an endogenous rodent C5 locus and the human C5 gene sequence comprising one or more exons of the human C5 gene sequence encoding human or humanized C5 protein is operably linked endogenous rodent regulatory elements or sequences at the endogenous rodent C5 locus.

34. A method for making a humanized mouse, comprising replacing a mouse C5

gene sequence encoding mouse C5 protein with a human C5 gene encoding human C5 protein.

35. The method of claim 34, wherein the replacement is at an endogenous mouse C5 locus, and the human C5 gene encoding human C5 protein is operably linked to endogenous mouse regulatory sequences at the endogenous mouse C5 locus.

36. A method for making a humanized rodent, comprising replacing a rodent C3 gene sequence encoding rodent C3 protein with a human C3 gene sequence comprising one or more exons of the human C3 gene sequence encoding human or humanized C5 protein, wherein the replacement is at an endogenous rodent C3 locus and the human C5 gene sequence comprising one or more exons of the human C3 gene sequence encoding human or humanized C3 protein is operably linked to rodent regulatory elements or sequences at the endogenous rodent C3 locus.

37. The method of claim 36, wherein the rodent is a mouse or a rat.

38. The method of claim 36 or 37, wherein the rodent regulatory elements or sequences are from a mouse or a rat.

39. The method of any one of claims 36-38, wherein the rodent regulatory elements or sequences are endogenous rodent regulatory elements or sequences at the rodent C3 locus.

40. The method of any one of claims 36-39, wherein the human C3 gene sequence replacing the rodent C5 gene sequence comprises at least one exon of the human C3 gene sequence.

41. The method of claim 40, wherein the human C3 gene sequence replacing the rodent C3 gene sequence comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 exons of the human C3 gene sequence.

42. The method of claim 41, wherein the human C3 gene sequence replacing the rodent C3 gene sequence comprises all 41 exons of the human C3 gene sequence.

43. The method of any one of claims 36-42, wherein the replacement is at an endogenous rodent C3 locus and the human C3 gene sequence comprising one or more exons of the human C3 gene sequence encoding human or humanized C3 protein is operably linked endogenous rodent regulatory elements or sequences at the endogenous rodent C3 locus.

44. A method for making a humanized mouse, comprising replacing a mouse C3 gene sequence encoding mouse C3 protein with a human C3 gene encoding human C3 protein.

45. The method of claim 44, wherein the replacement is at an endogenous mouse C3 locus, and the human C3 gene encoding human C3 protein is operably linked to endogenous mouse regulatory sequences at the endogenous mouse C3 locus.

46. A rodent, comprising a humanized C5 gene comprising a replacement at an endogenous rodent C5 locus of a rodent gene encoding C5 protein with a human C5 gene encoding human or humanized C5 protein, wherein expression of the human C5 gene encoding human or humanized C5 protein is under control of rodent regulatory elements or sequences at the endogenous rodent C5 locus, and wherein the rodent further comprises a replacement at an endogenous rodent C3 locus of a rodent gene encoding C3 protein with a human C3 gene encoding human or humanized C3 protein,

wherein expression of the human C3 gene encoding human or humanized C3 protein is under control of rodent regulatory elements or sequences at the endogenous rodent C3 locus.

47. The rodent of claim 46, wherein the rodent is a mouse or a rat.

48. The rodent of claim 46, wherein the rodent is a mouse that is incapable of expressing a mouse C5 protein and incapable of expressing a mouse C3 protein.

49. The rodent of claim 46, wherein the rodent regulatory elements or sequences at the endogenous rodent C5 locus and/or endogenous rodent C3 locus are from a mouse or a rat.

50. The rodent of claim 46, wherein the rodent regulatory elements or sequences are endogenous rodent regulatory elements or sequences at the rodent C5 locus and/or rodent C3 locus.

51. A method for identifying a compound capable of modulating complement activation comprising:

a. administering the compound to the rodent of any one of claims 1-25 or 46-50; and
b. assaying if complement activation in the rodent is modulated, thereby identifying a compound capable of modulating complement activation.

52. The method of claim 51, wherein the compound is a small molecule chemical compound, an antibody, a protein, an inhibitory nucleic acid, or any combination thereof.

53. The method of claim 51 or claim 52, wherein the compound modulates

complement activation by increasing complement activity.

54. The method of claim 51 or claim 52, wherein the compound modulates complement activation by decreasing complement activity.

55. The method of any one of claims 51-54, wherein the serum of the rodent is assayed to determine if complement activation in the rodent is modulated.

56. The method of claim 55, wherein the assay is the CH_{50} complement screening assay.

AMENDED CLAIMS
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1. A rodent, comprising a replacement at an endogenous rodent C5 locus of a rodent gene sequence encoding an exon of a C5 gene with a nucleic acid sequence encoding at least one exon of a human C5 gene to form a modified C5 gene, wherein expression of the modified C5 gene is under control of rodent regulatory elements at the endogenous rodent C5 locus, wherein the human C5 gene encoding human C5 protein comprises exon 2 through exon 41 of the human C5 gene.

2. The rodent of claim 1, wherein the rodent is a mouse or a rat.

3. The rodent of claim 1 or claim 2, wherein the rodent is a mouse that is incapable of expressing a mouse C5 protein.

4. The rodent of any one of claims 1-3, wherein the rodent is a mouse that expresses a mouse C3 protein encoded by an endogenous mouse C3 gene.

5. The rodent of any one of claims 1-4, wherein the rodent is a mouse that expresses a human or humanized C3 protein.

6. The rodent of claim 5, wherein the mouse comprises a replacement at an endogenous mouse C3 locus of a mouse gene encoding C3 protein with a human C3 gene encoding a human C3 protein.

7. The rodent of claim 5 or claim 6, wherein expression of the human C3 gene is under control of mouse regulatory elements at the endogenous mouse C3 locus.

8. (Currently amended) The rodent of any one of claims 1-7, wherein the rodent is a mouse that expresses human C5 protein in serum at a concentration of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%,

170%, 180%, 190% or 200% of the level of mouse C5 protein present in the serum of an age-matched mouse that expresses functional endogenous mouse C5 protein, but does not comprise said replacement.

9. The rodent of any one of claims 1-8, wherein the rodent is a mouse that expresses human C5 protein in serum at a concentration of between about 10% and about 200%, between about 20% and about 150%, or between about 30% and about 100% of the level of mouse C5 protein present in the serum of an age-matched mouse that expresses functional endogenous C5 protein, but does not comprise said replacement.

10. The rodent of any one of claims 1-9, wherein the rodent is a mouse that expresses human C5 protein in serum at a concentration of between about 10 μg/ml and about 150 μg/ml, between about 10 μg/ml and about 125 μg/ml, or between about 15 μg/ml and about 100 μg/ml.

11. The rodent of any one of claims 1-10, wherein the rodent is a mouse that expresses human C5 protein in serum at a concentration of at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125 or 150 μg/ml.

12. A rodent, comprising a replacement at an endogenous rodent C3 locus of a rodent gene sequence encoding an exon of a C3 gene with a nucleic acid sequence encoding at least one exon of a human C3 gene to form a modified C3 gene, wherein expression of the modified C3 gene is under control of rodent regulatory elements at the endogenous rodent C3 locus.

13. The rodent of claim 12, wherein the rodent is a mouse or a rat

14. The rodent of claim 12 or claim 13, wherein the human C3 gene encoding human C3 protein comprises exon 1 through exon 41 of the human C3 gene.

15. The rodent of any one of claims 12-14, wherein the human C3 gene encoding human C3

protein comprises exon 2 through exon 41 of the human C3 gene.

16. The rodent of any one of claims 12-15, wherein the rodent is a mouse that is incapable of expressing a mouse C3 protein.

17. The rodent of any one of claims 12-16, wherein the rodent is a mouse that expresses a mouse C5 protein encoded by an endogenous mouse C5 gene.

18. The rodent of claim 17 or claim 18, wherein the mouse expresses a human or humanized C5 protein.

19. The rodent of claim 18, wherein the mouse comprises a replacement at an endogenous mouse C5 locus of a mouse gene encoding C5 protein with a human C5 gene encoding human C5 protein.

20. The rodent of claim 18 or claim 19, wherein expression of the human C5 gene is under control of mouse regulatory elements at the endogenous mouse C5 locus.

21. The rodent of any one of claims 12-20, wherein the rodent is a mouse that expresses human C3 protein in serum at a concentration of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190% or 200% of the level of mouse C3 protein present in the serum of an age-matched mouse that expresses functional endogenous mouse C3 protein, but does not comprise said replacement.

22. The rodent of any one of claims 12-21, wherein the rodent is a mouse that expresses human C3 protein in serum at a concentration of between about 10% and about 200%, between about 20% and about 150%, or between about 30% and about 100% of the level of mouse C3 protein present in the serum of an age-matched mouse that expresses functional endogenous mouse C3 protein, but does not comprise said replacement.

23. The rodent of any one of claims 12-22, wherein the rodent is a mouse that expresses human C3 protein in serum at a concentration of between about 100 μg/ml and about 1500 μg/ml, between about 200 μg/ml and about 1250 μg/ml, or between about 300 μg/ml and about 1000 μg/ml.

24. The rodent of any one of claims 12-23, wherein the rodent is a mouse that expresses human C3 protein in serum at a concentration of at least about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1250 or 1500 μg/ml.

25. A method for making a humanized rodent, comprising replacing a rodent C5 gene sequence encoding rodent C5 protein with a human C5 gene sequence comprising one or more exons of the human C5 gene sequence encoding human or humanized C5 protein, wherein the replacement is at an endogenous rodent C5 locus and the human C5 gene sequence comprising one or more exons of the human C5 gene sequence encoding human or humanized C5 protein is operably linked to rodent regulatory elements or sequences at the endogenous rodent C5 locus, wherein the rodent regulatory elements or sequences are from a mouse or a rat.

26. The method of claim 25, wherein the rodent is a mouse or a rat.

27. The method of claim 25 or claim 26, wherein the rodent regulatory elements or sequences are endogenous rodent regulatory elements or sequences at the rodent C5 locus.

28. The method of any one of claims 25-27, wherein the human C5 gene sequence replacing the rodent C5 gene sequence comprises at least one exon of the human C5 gene sequence.

29. The method of claim 28, wherein the human C5 gene sequence replacing the rodent C5 gene sequence comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 exons of the human

C5 gene sequence.

30. The method of claim 28 or claim 29, wherein the human C5 gene sequence replacing the rodent C5 gene sequence comprises all 41 exons of the human C5 gene sequence.

31. The method of any one of claims 25-30, wherein the replacement is at an endogenous rodent C5 locus and the human C5 gene sequence comprising one or more exons of the human C5 gene sequence encoding human or humanized C5 protein is operably linked endogenous rodent regulatory elements or sequences at the endogenous rodent C5 locus.

32. A method for making a humanized mouse, comprising replacing a mouse C5 gene sequence encoding mouse C5 protein with a human C5 gene encoding human C5 protein, wherein the replacement is at an endogenous mouse C5 locus, and the human C5 gene encoding human C5 protein is operably linked to endogenous mouse regulatory sequences at the endogenous mouse C5 locus.

33. A method for making a humanized rodent, comprising replacing a rodent C3 gene sequence encoding rodent C3 protein with a human C3 gene sequence comprising one or more exons of the human C3 gene sequence encoding human or humanized C3 protein, wherein the replacement is at an endogenous rodent C3 locus and the human C3 gene sequence comprising one or more exons of the human C3 gene sequence encoding human or humanized C3 protein is operably linked to rodent regulatory elements or sequences at the endogenous rodent C3 locus.

34. The method of claim 33, wherein the rodent is a mouse or a rat.

35. The method of claim 33 or 34, wherein the rodent regulatory elements or sequences are from a mouse or a rat.

36. The method of any one of claims 33-35, wherein the rodent regulatory elements or sequences are endogenous rodent regulatory elements or sequences at the rodent C3 locus.

37. The method of any one of claims 33-36, wherein the human C3 gene sequence replacing the rodent C3 gene sequence comprises at least one exon of the human C3 gene sequence.

38. The method of claim 37, wherein the human C3 gene sequence replacing the rodent C3 gene sequence comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 exons of the human C3 gene sequence.

39. The method of claim 38, wherein the human C3 gene sequence replacing the rodent C3 gene sequence comprises all 41 exons of the human C3 gene sequence.

40. The method of any one of claims 33-39, wherein the replacement is at an endogenous rodent C3 locus and the human C3 gene sequence comprising one or more exons of the human C3 gene sequence encoding human or humanized C3 protein is operably linked endogenous rodent regulatory elements or sequences at the endogenous rodent C3 locus.

41. A method for making a humanized mouse, comprising replacing a mouse C3 gene sequence encoding mouse C3 protein with a human C3 gene encoding human C3 protein.

42. The method of claim 41, wherein the replacement is at an endogenous mouse C3 locus, and the human C3 gene encoding human C3 protein is operably linked to endogenous mouse regulatory sequences at the endogenous mouse C3 locus.

43. A rodent, comprising a humanized C5 gene comprising a replacement at an endogenous rodent C5 locus of a rodent gene encoding C5 protein with a human C5 gene encoding human or humanized C5 protein, wherein expression of the human C5 gene encoding human or humanized C5 protein is under control of rodent regulatory elements or sequences at the endogenous rodent C5 locus, and wherein the rodent further comprises a replacement at an endogenous rodent C3 locus of a rodent gene encoding C3 protein with a human C3 gene encoding human or

humanized C3 protein, wherein expression of the human C3 gene encoding human or humanized C3 protein is under control of rodent regulatory elements or sequences at the endogenous rodent C3 locus.

44. The rodent of claim 43, wherein the rodent is a mouse or a rat.

45. The rodent of claim 43, wherein the rodent is a mouse that is incapable of expressing a mouse C5 protein and incapable of expressing a mouse C3 protein.

46. The rodent of claim 43, wherein the rodent regulatory elements or sequences at the endogenous rodent C5 locus and/or endogenous rodent C3 locus are from a mouse or a rat.

47. The rodent of claim 43, wherein the rodent regulatory elements or sequences are endogenous rodent regulatory elements or sequences at the rodent C5 locus and/or rodent C3 locus.

48. A method for identifying a compound capable of modulating complement activation comprising:

a. administering the compound to the rodent of any one of claims 1-24 or 43-47; and

b. assaying if complement activation in the rodent is modulated, thereby identifying a compound capable of modulating complement activation.

49. The method of claim 48, wherein the compound is a small molecule chemical compound, an antibody, a protein, an inhibitory nucleic acid, or any combination thereof.

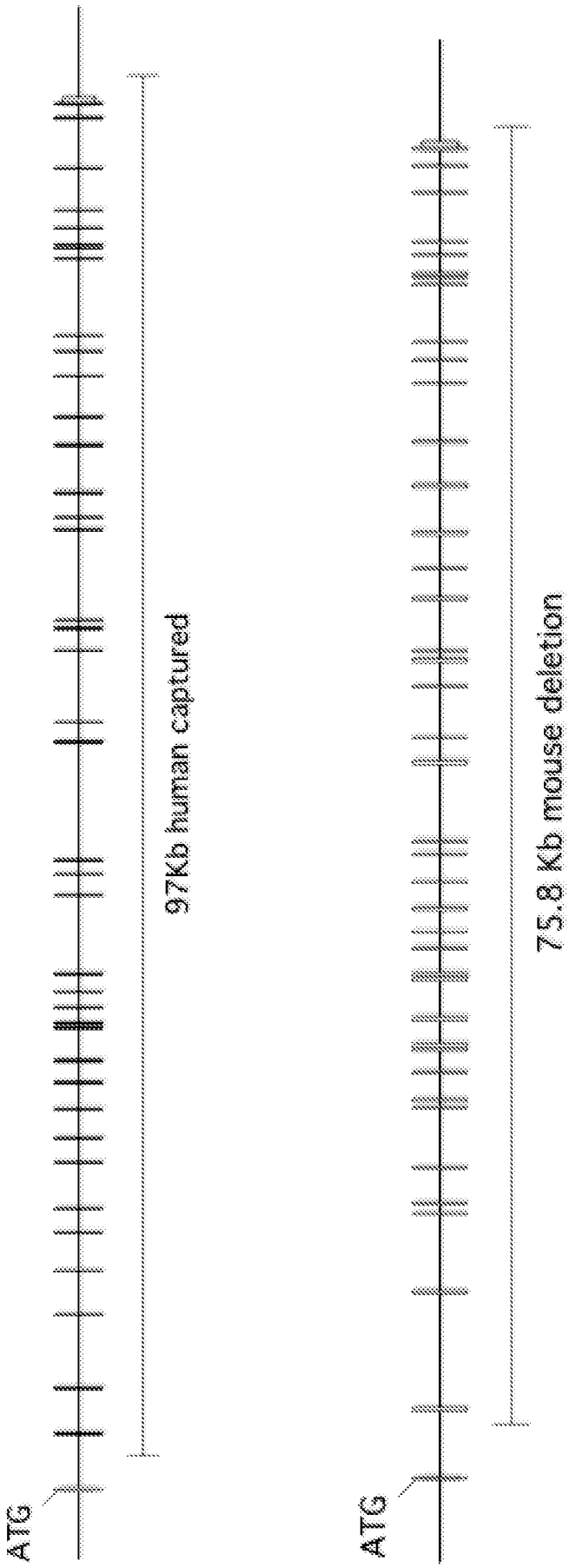
50. The method of claim 48 or claim 49, wherein the compound modulates complement activation by increasing complement activity.

51. The method of claim 48 or claim 49, wherein the compound modulates complement activation by decreasing complement activity.

52. The method of any one of claims 48-51, wherein the serum of the rodent is assayed to determine if complement activation in the rodent is modulated.

53. The method of claim 52, wherein the assay is the CH_{50} complement screening assay.

FIGURE 1



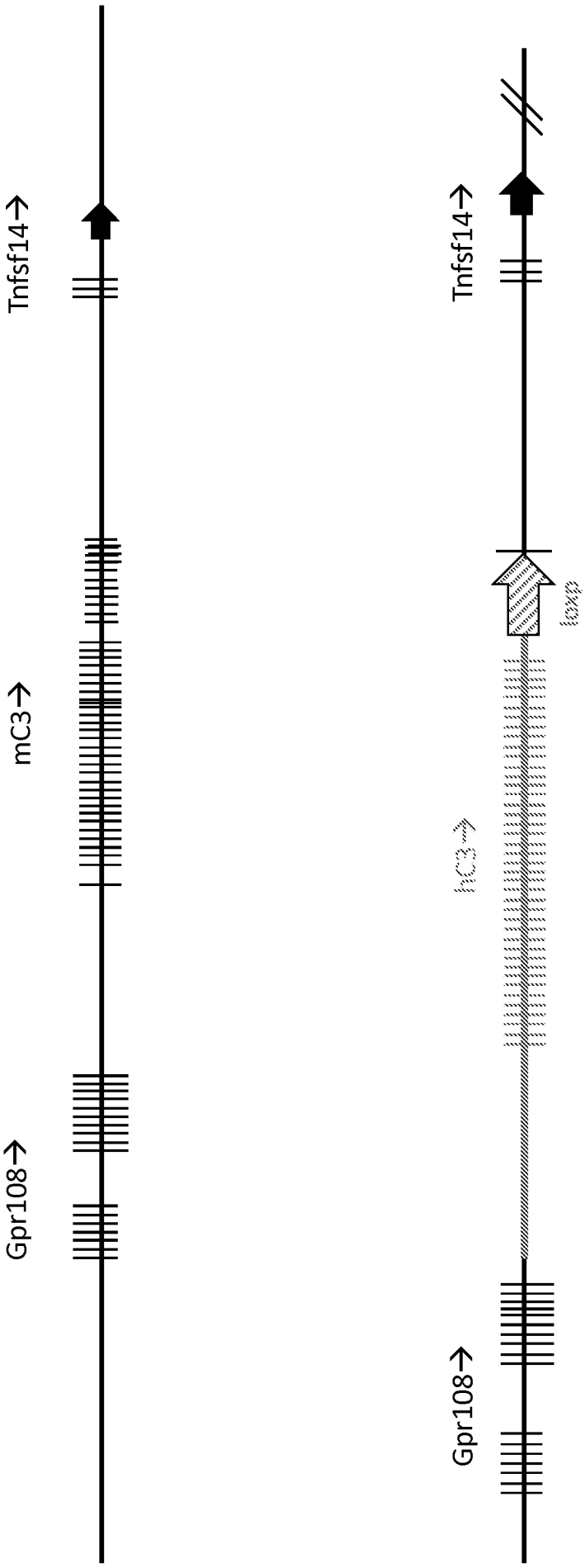


FIGURE 2A

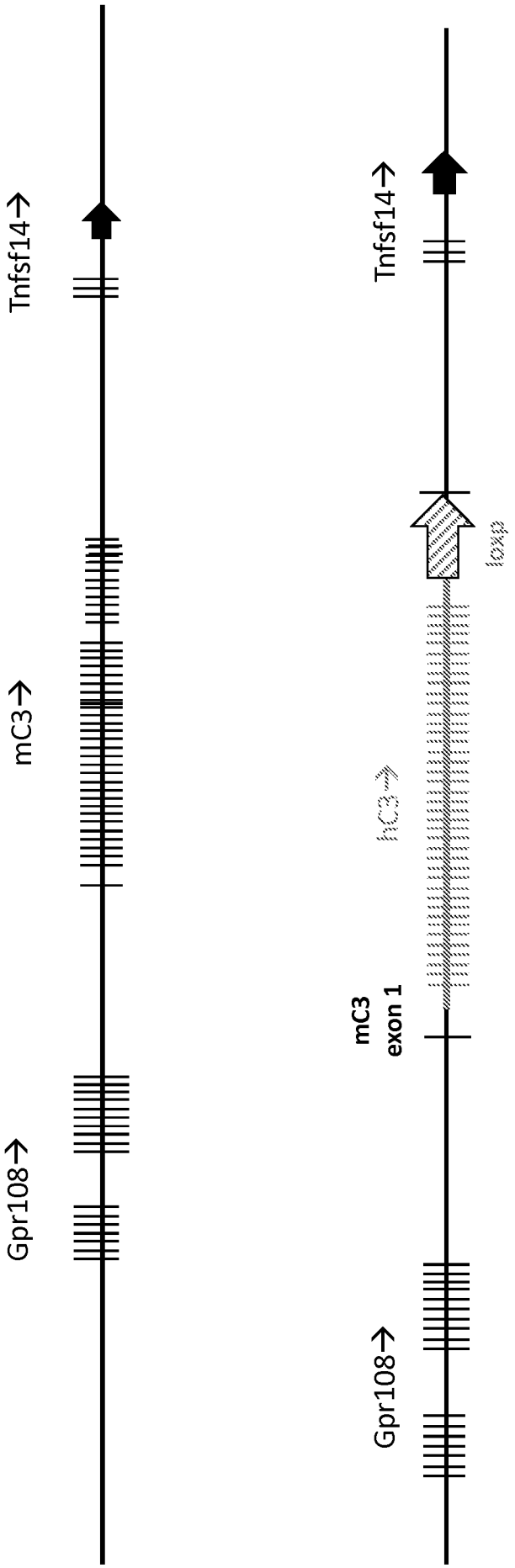
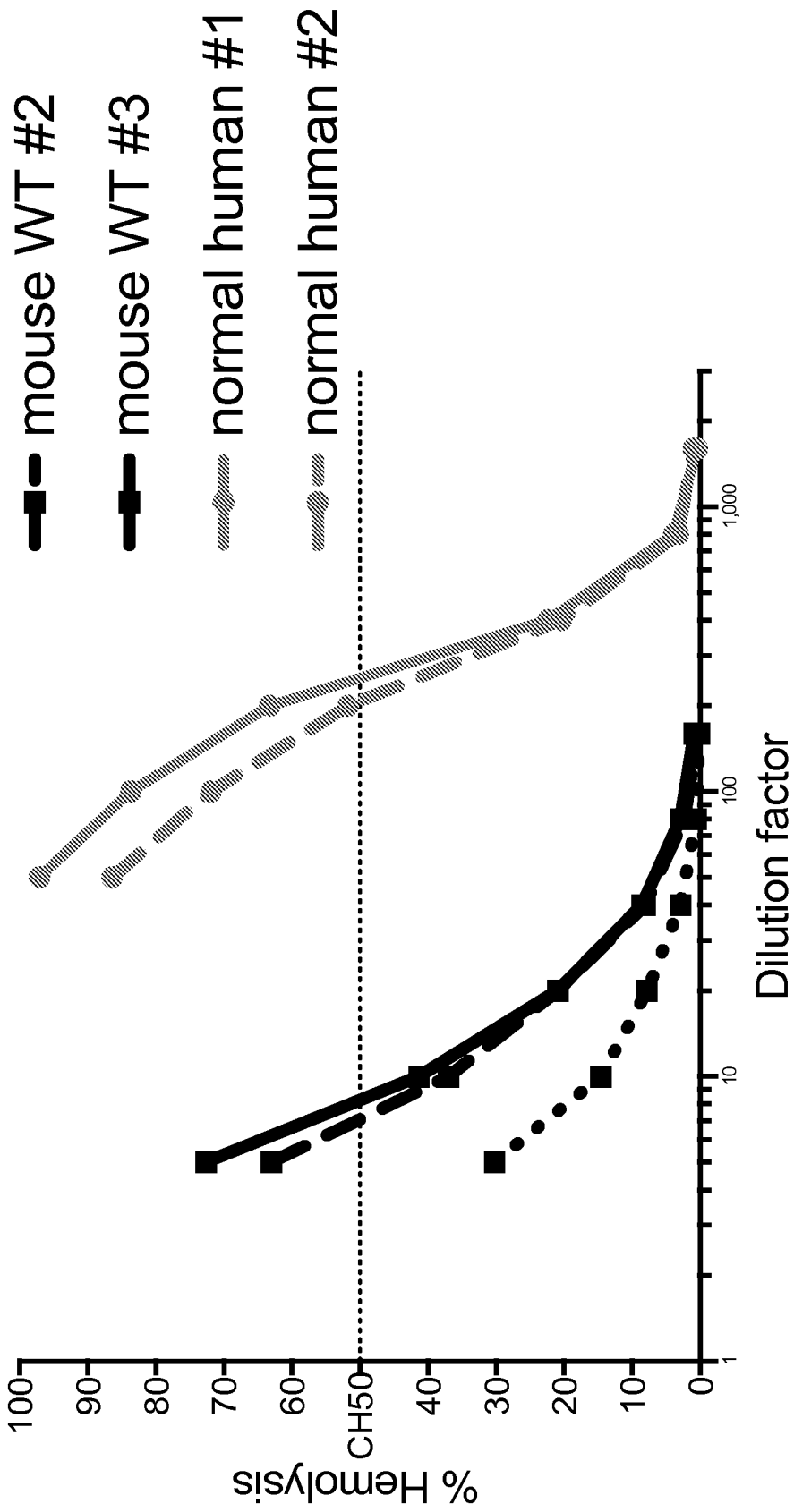


FIGURE 2B

FIGURE 3


mouse WT #1
mouse WT #2
mouse WT #3
normal human #1
normal human #2
% Hemolysis
CH50
Dilution factor
100
90
80
70
60
40
30
20
10
0
1
10
100
1,000

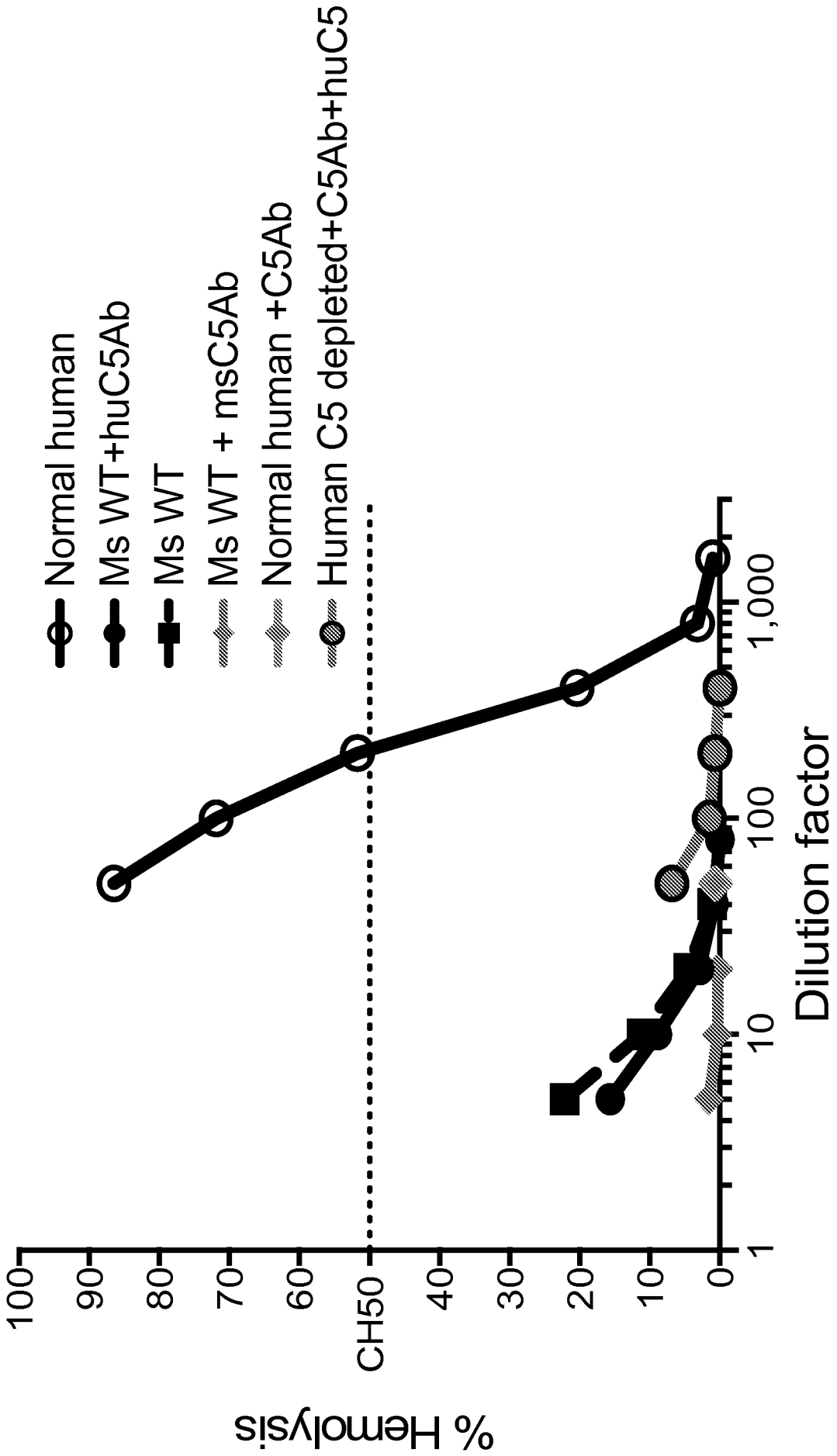

Normal human
Ms WT+huC5Ab
Ms WT
Ms WT + msC5Ab
Normal human +C5Ab
Human C5 depleted+C5Ab+huC5
% Hemolysis
100
90
80
70
60
CH50
40
30
20
10
0
1
10
100
1,000
Dilution factor

FIGURE 4

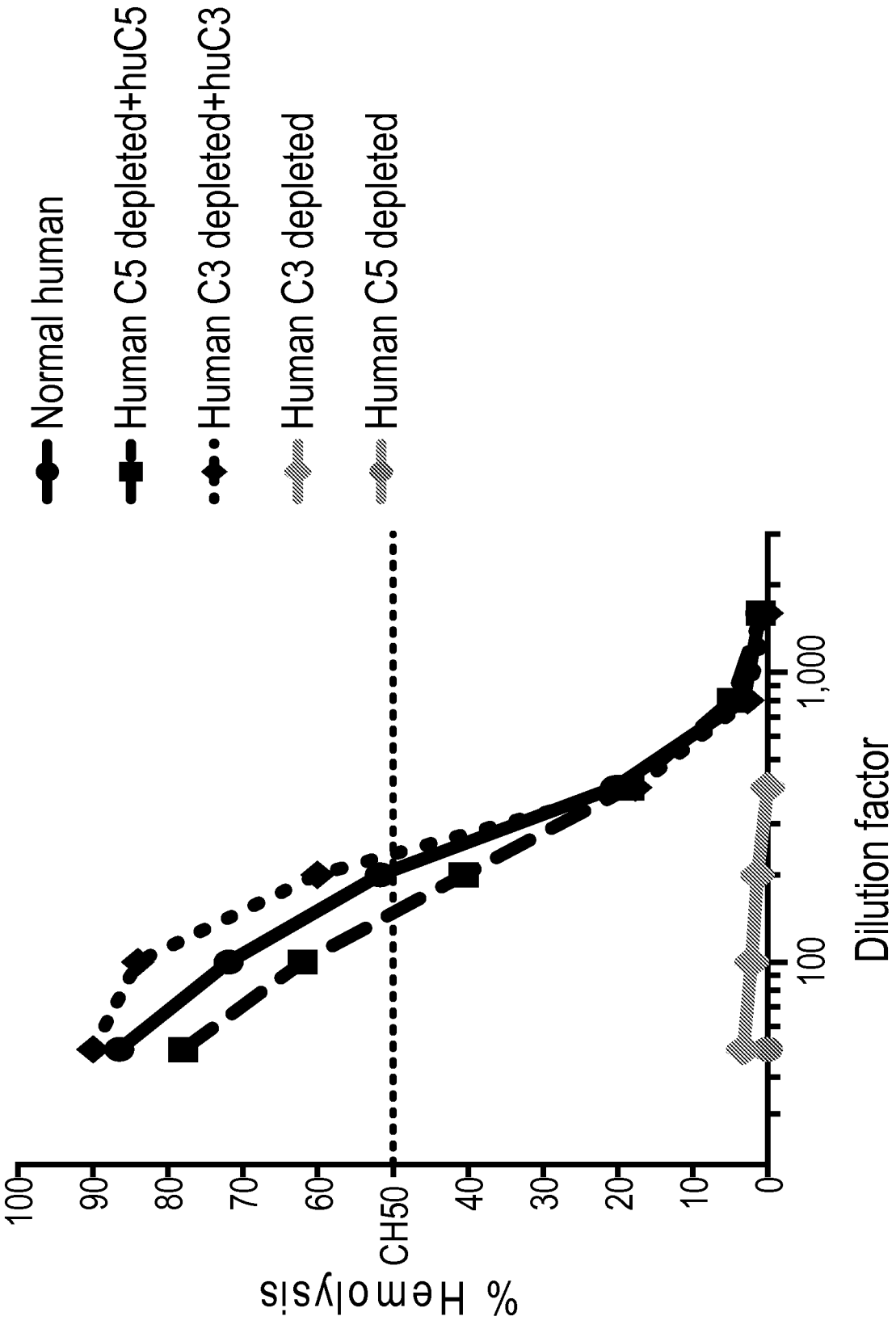

Normal human
Human C5 depleted+huC5
Human C3 depleted+huC3
Human C3 depleted
Human C5 depleted
% Hemolysis
CH50
100
90
80
70
60
40
30
20
10
0
100
1,000
Dilution factor

FIGURE 5A

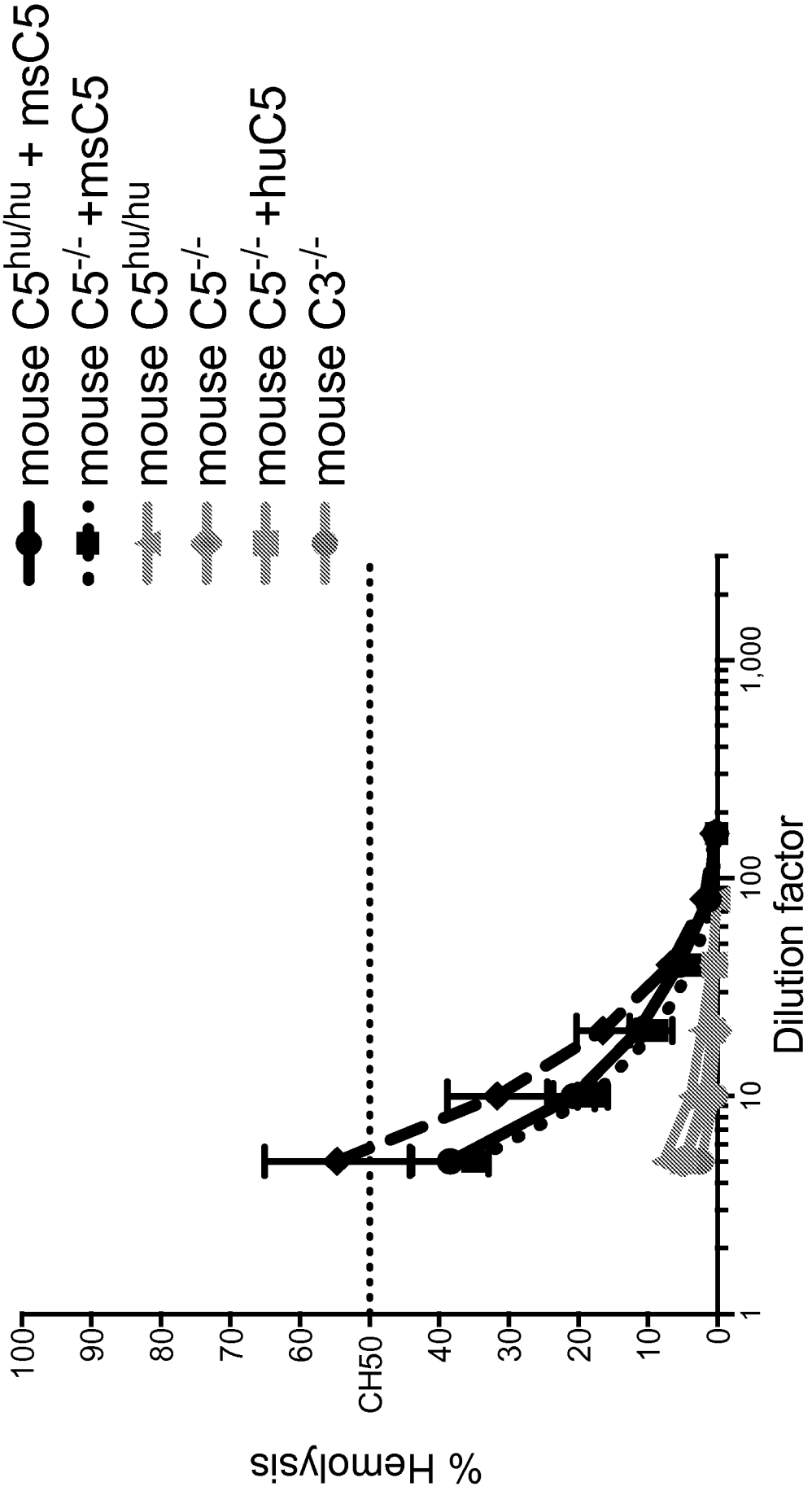

mouse C5WT/WT
mouse C5hu/hu + msC5
mouse C5-/- +msC5
mouse C5hu/hu
mouse C5-/-
mouse C5-/- +huC5
mouse C3-/-
% Hemolysis
CH50
100
90
80
70
60
40
30
20
10
0
1
10
100
1,000
Dilution factor

FIGURE 5B

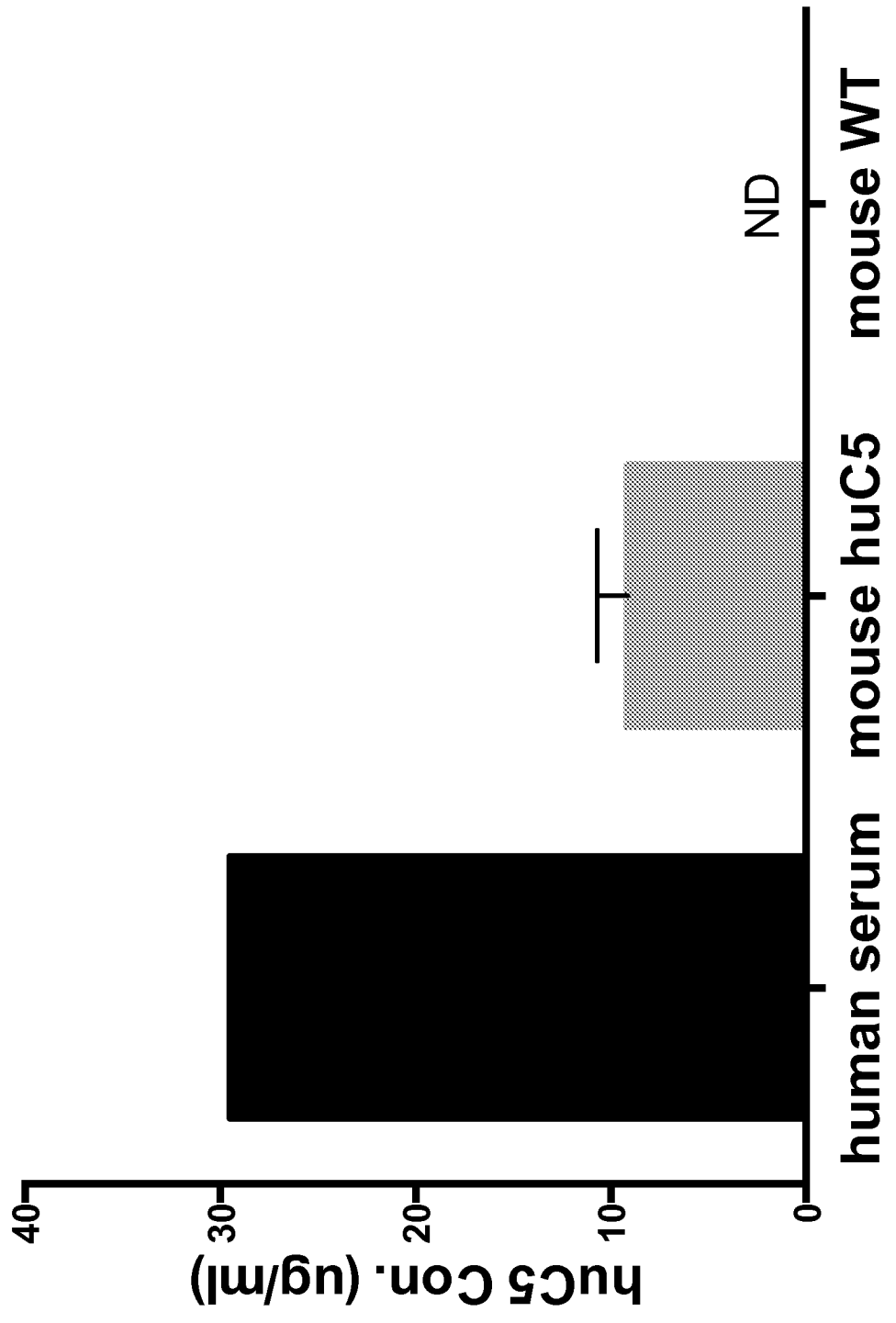


FIGURE 5C

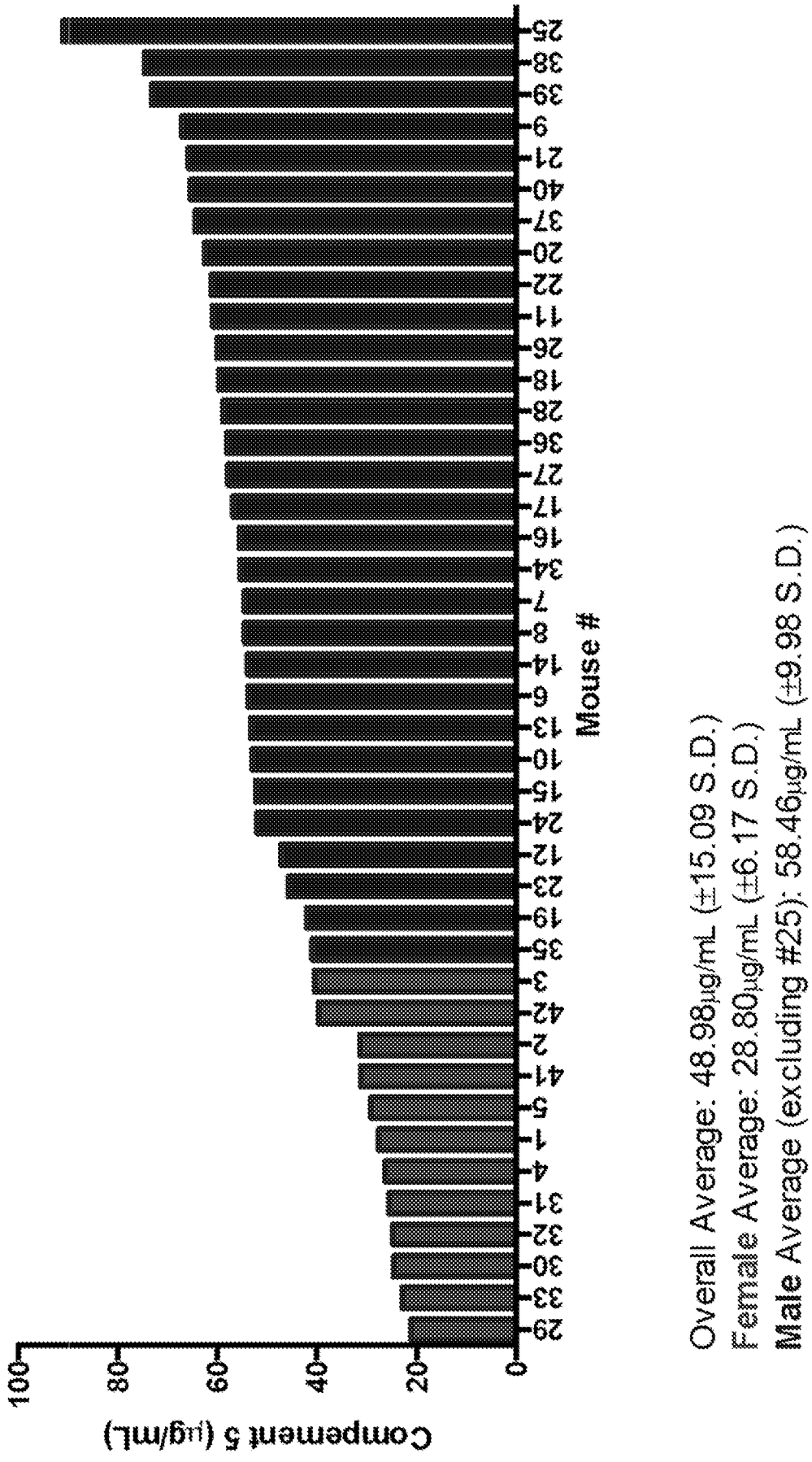

Pre-Dose C5 Levels in Mice Expressing Human C5
Complement 5 (µg/mL)
0
20
40
60
80
100
Mouse #
29
33
30
32
31
4
1
5
41
2
42
3
35
19
23
12
24
15
10
13
6
14
8
7
34
16
17
27
36
28
18
26
11
22
20
37
40
21
9
39
38
25
Overall Average: 48.98µg/mL (±15.09 S.D.)
Female Average: 28.80µg/mL (±6.17 S.D.)
Male Average (excluding #25): 58.46µg/mL (±9.98 S.D.)

FIGURE 5D

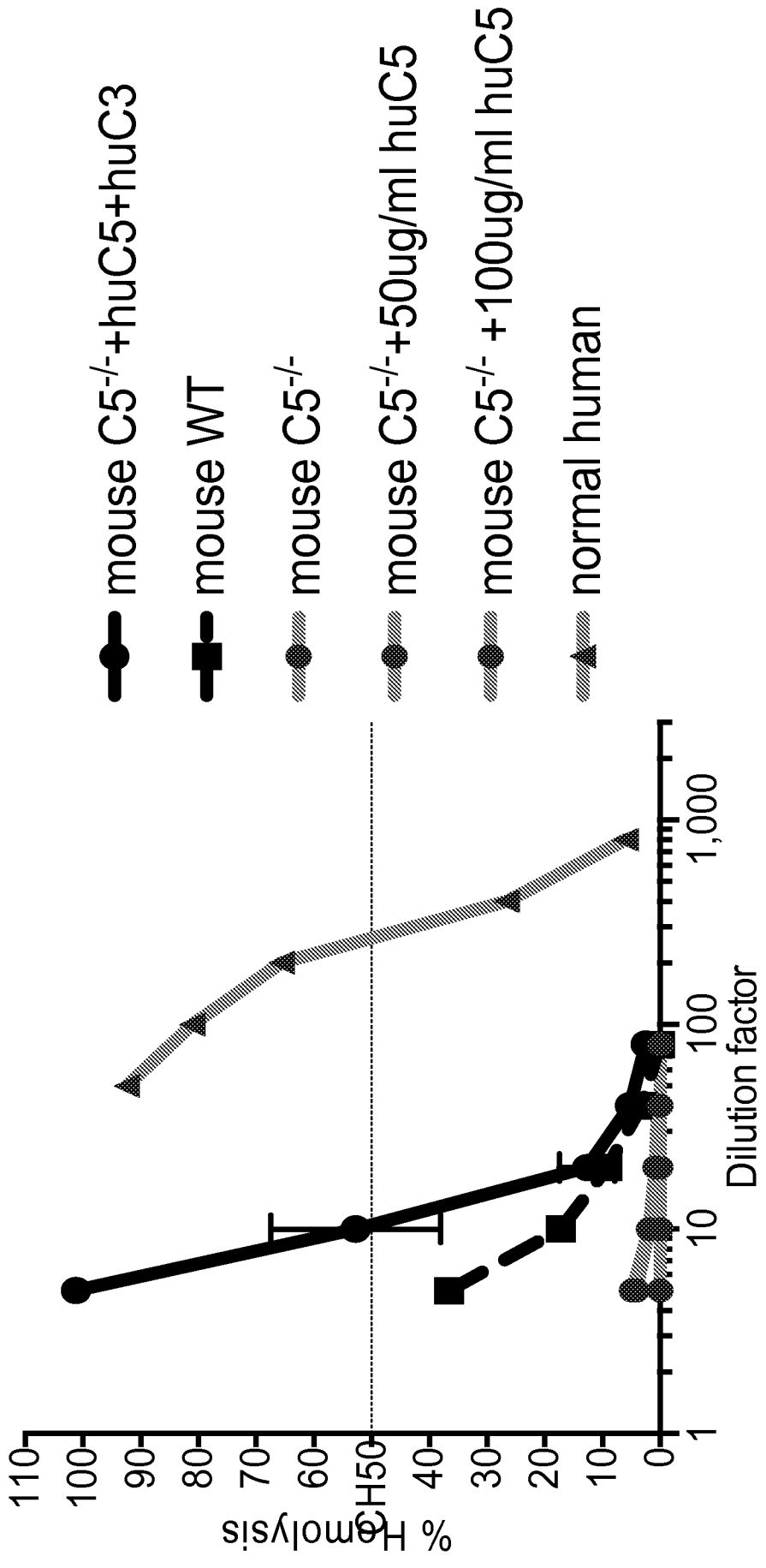

mouse C5-/-+huC5+huC3
mouse WT
mouse C5-/-
mouse C5-/-+50ug/ml huC5
mouse C5-/- +100ug/ml huC5
normal human
% Hemolysis (CH50)
110
100
90
80
70
60
50
40
30
20
10
0
1
10
100
1,000
Dilution factor

FIGURE 6

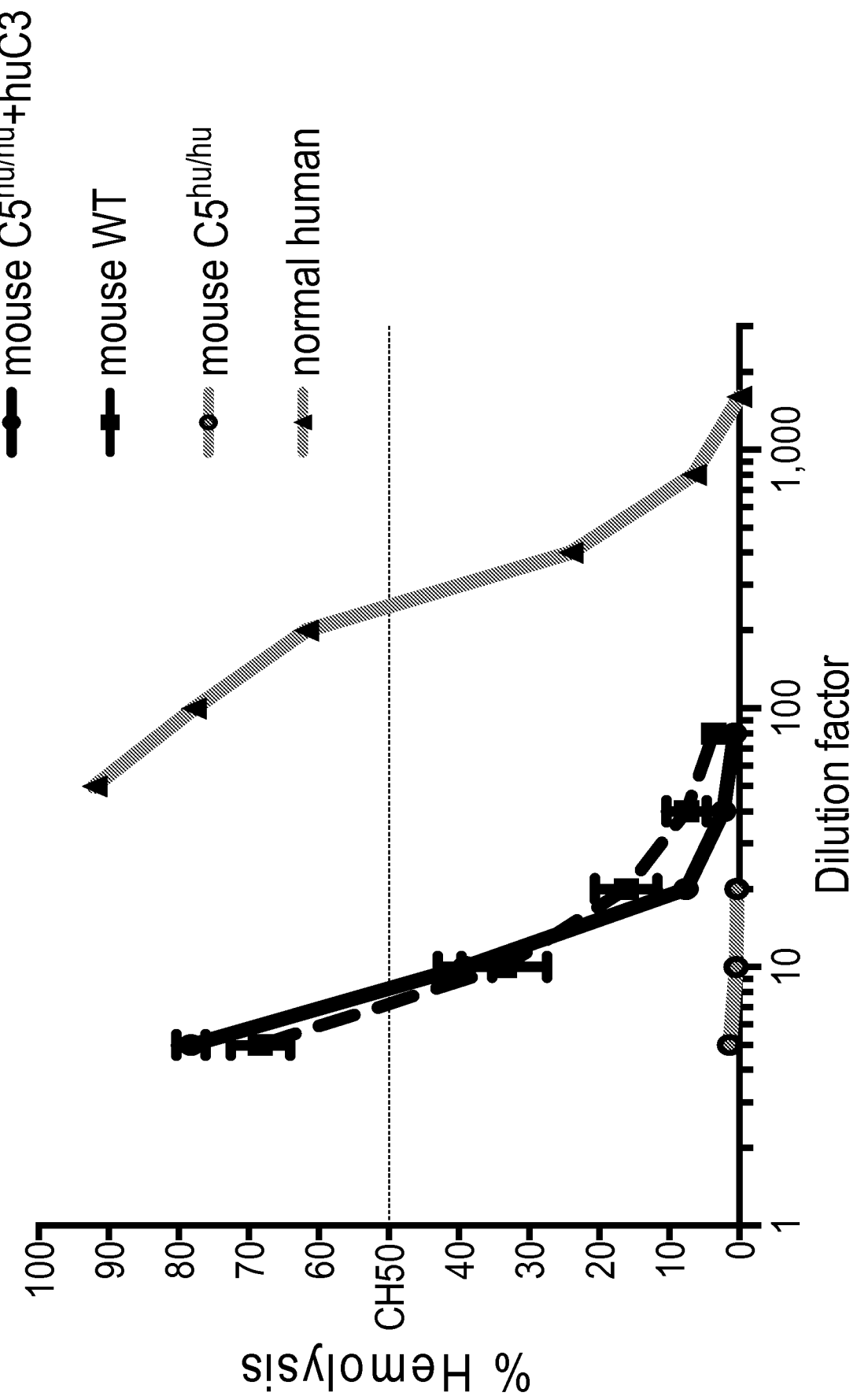

mouse C5hu/hu+huC3
mouse WT
mouse C5hu/hu
normal human
% Hemolysis
CH50
Dilution factor
100
90
80
70
60
40
30
20
10
0
1
10
100
1,000

FIGURE 7

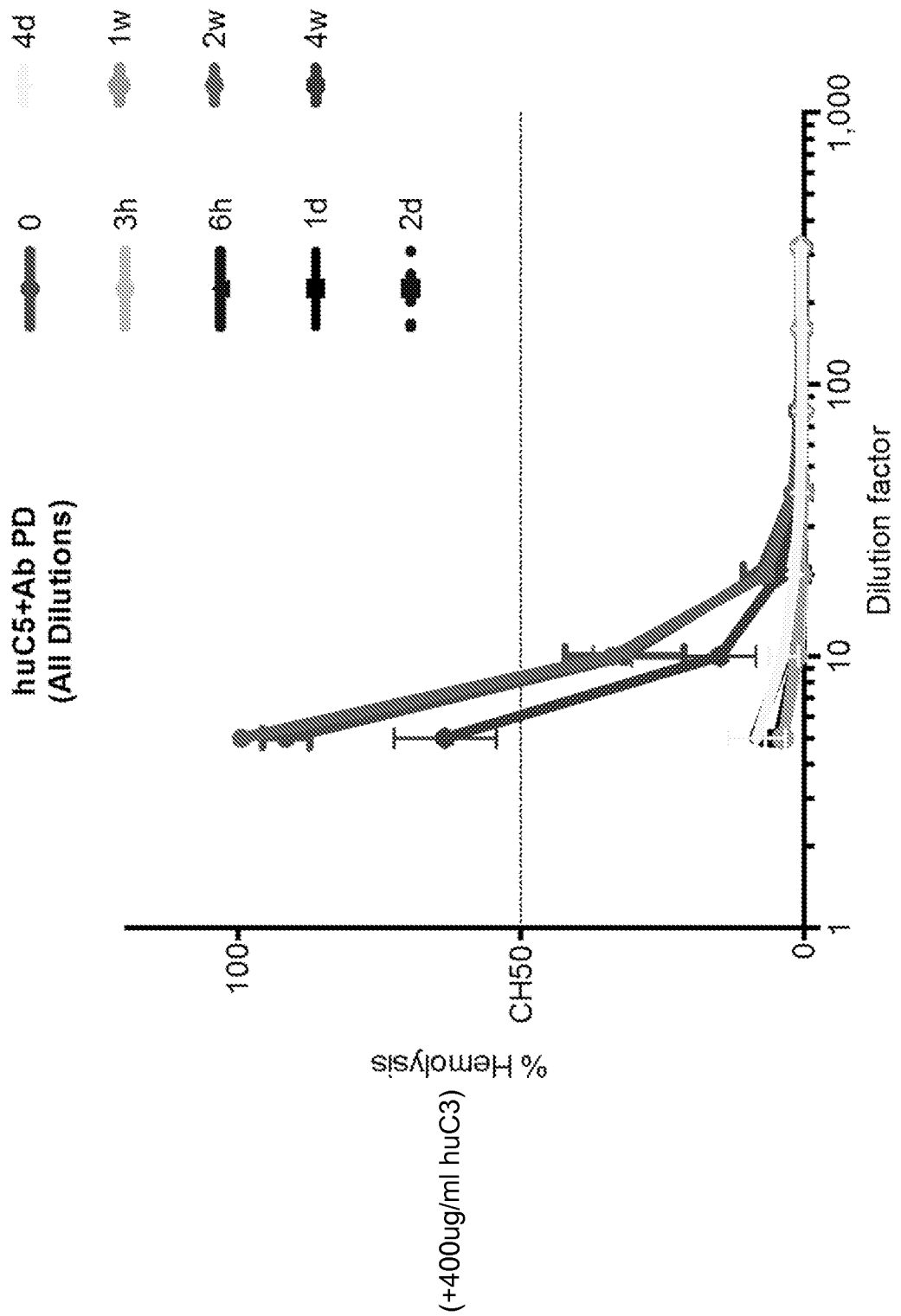

huC5+Ab PD
(All Dilutions)
0
3h
6h
1d
2d
4d
1w
2w
4w
% Hemolysis
(+400ug/ml huC3)
100
CH50
0
1
10
100
1,000
Dilution factor

FIGURE 8A

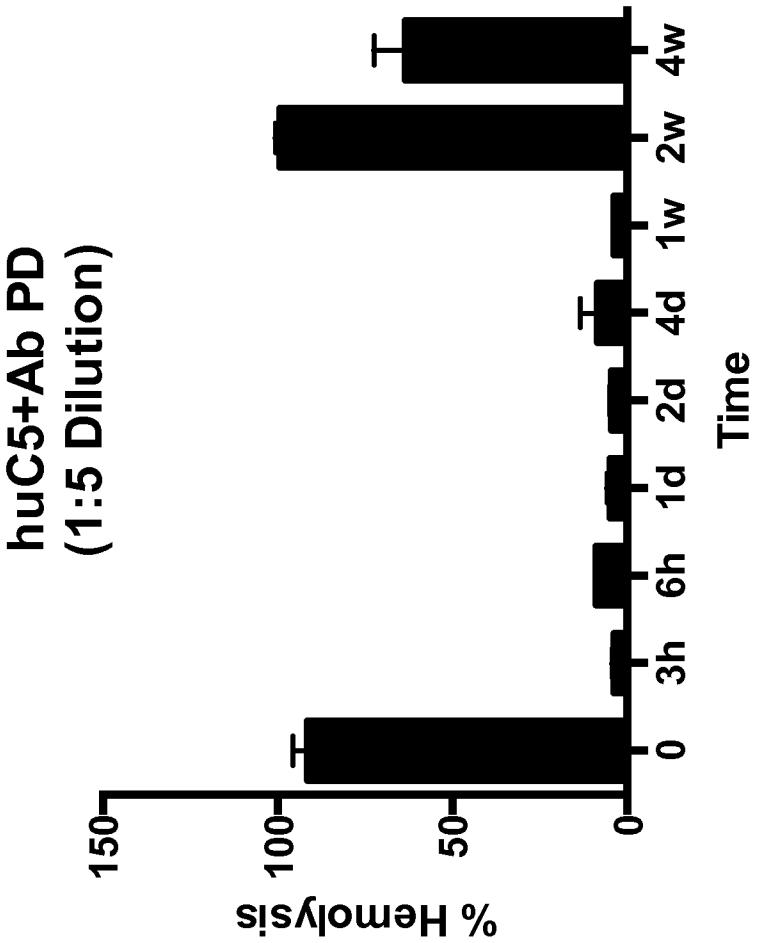


FIGURE 8A (CONT)

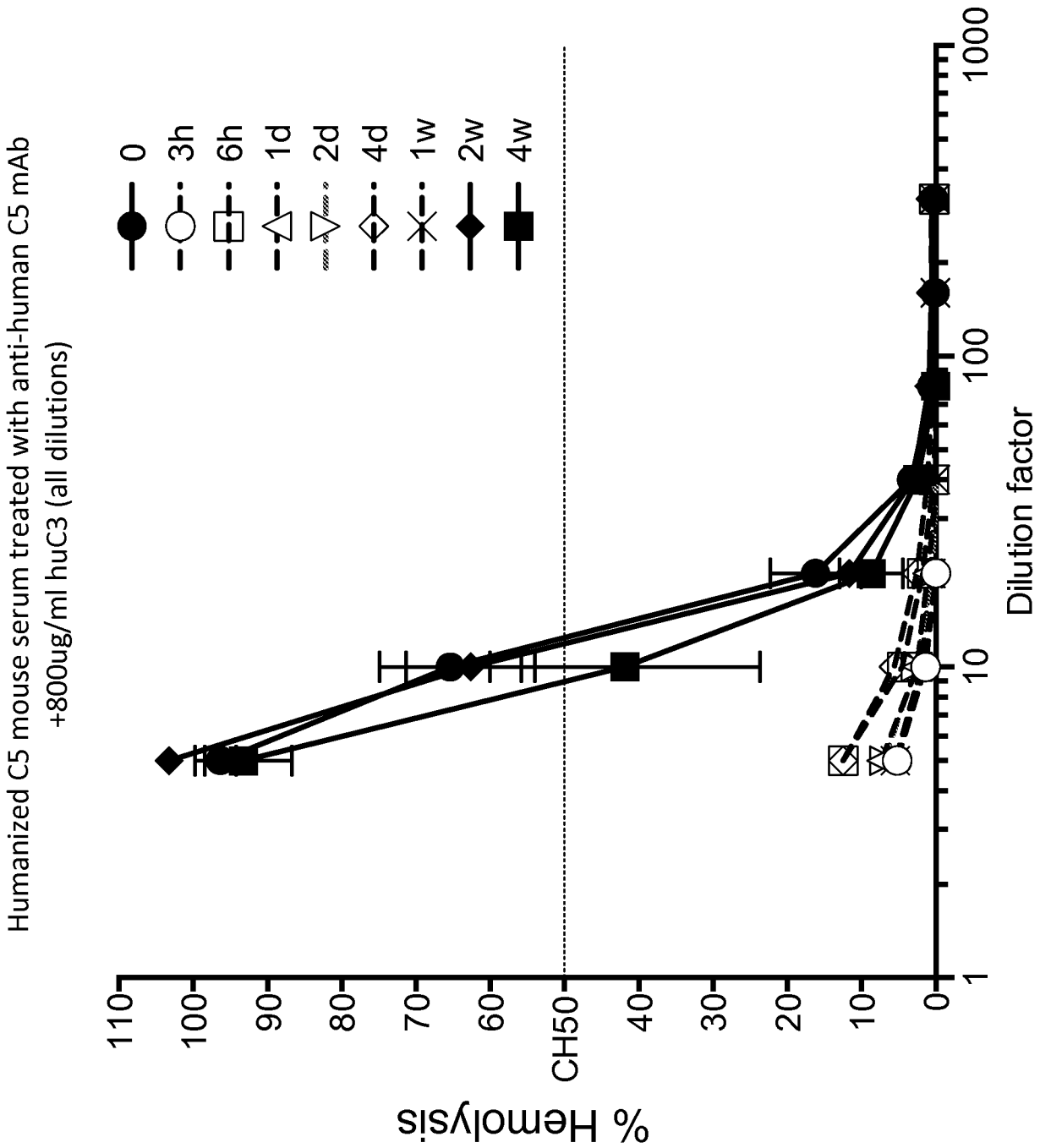

Humanized C5 mouse serum treated with anti-human C5 mAb
+800ug/ml huC3 (all dilutions)
0
3h
6h
1d
2d
4d
1w
2w
4w
% Hemolysis
CH50
110
100
90
80
70
60
40
30
20
10
0
1
10
100
1000
Dilution factor

FIGURE 8B

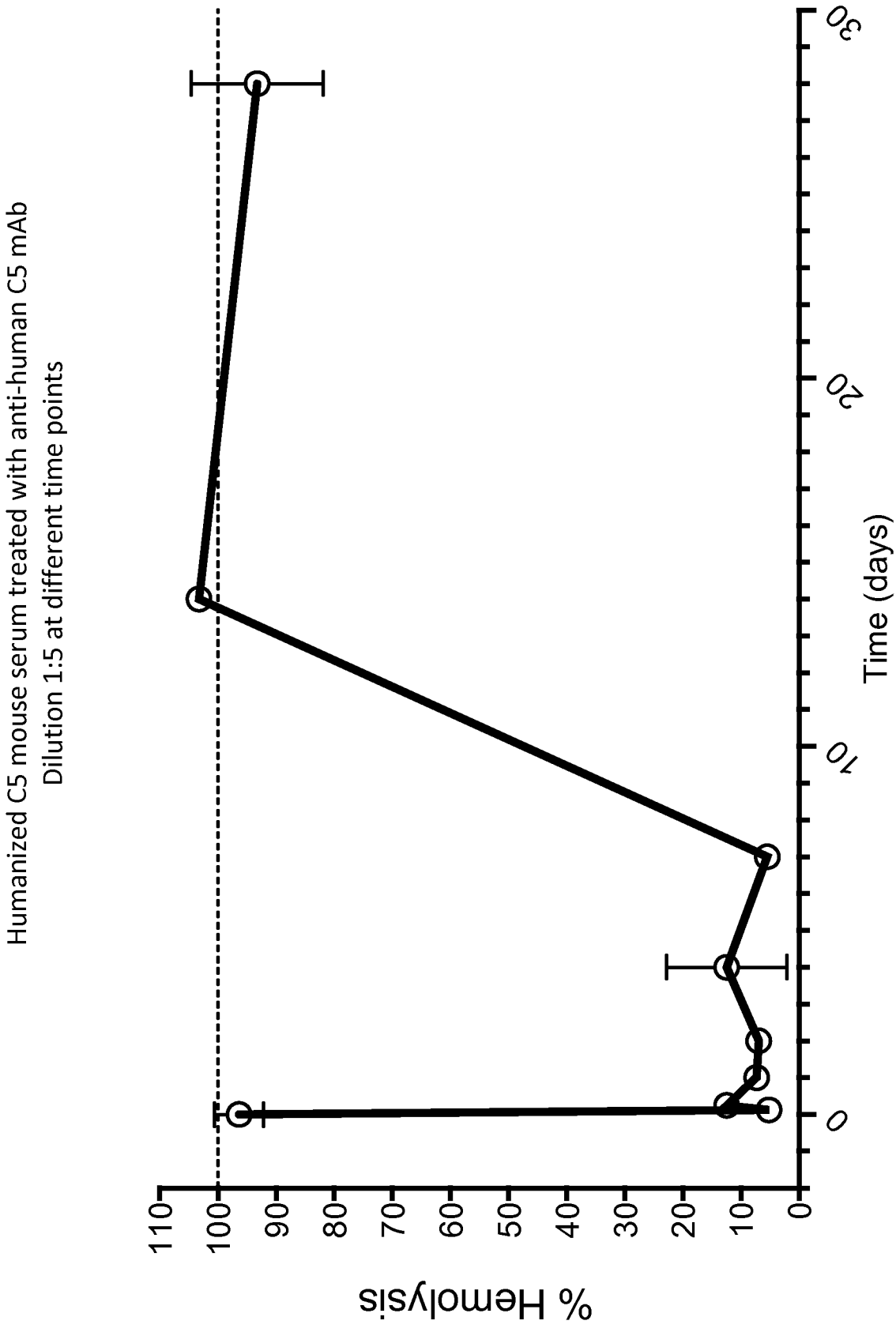


FIGURE 8B (CONT)

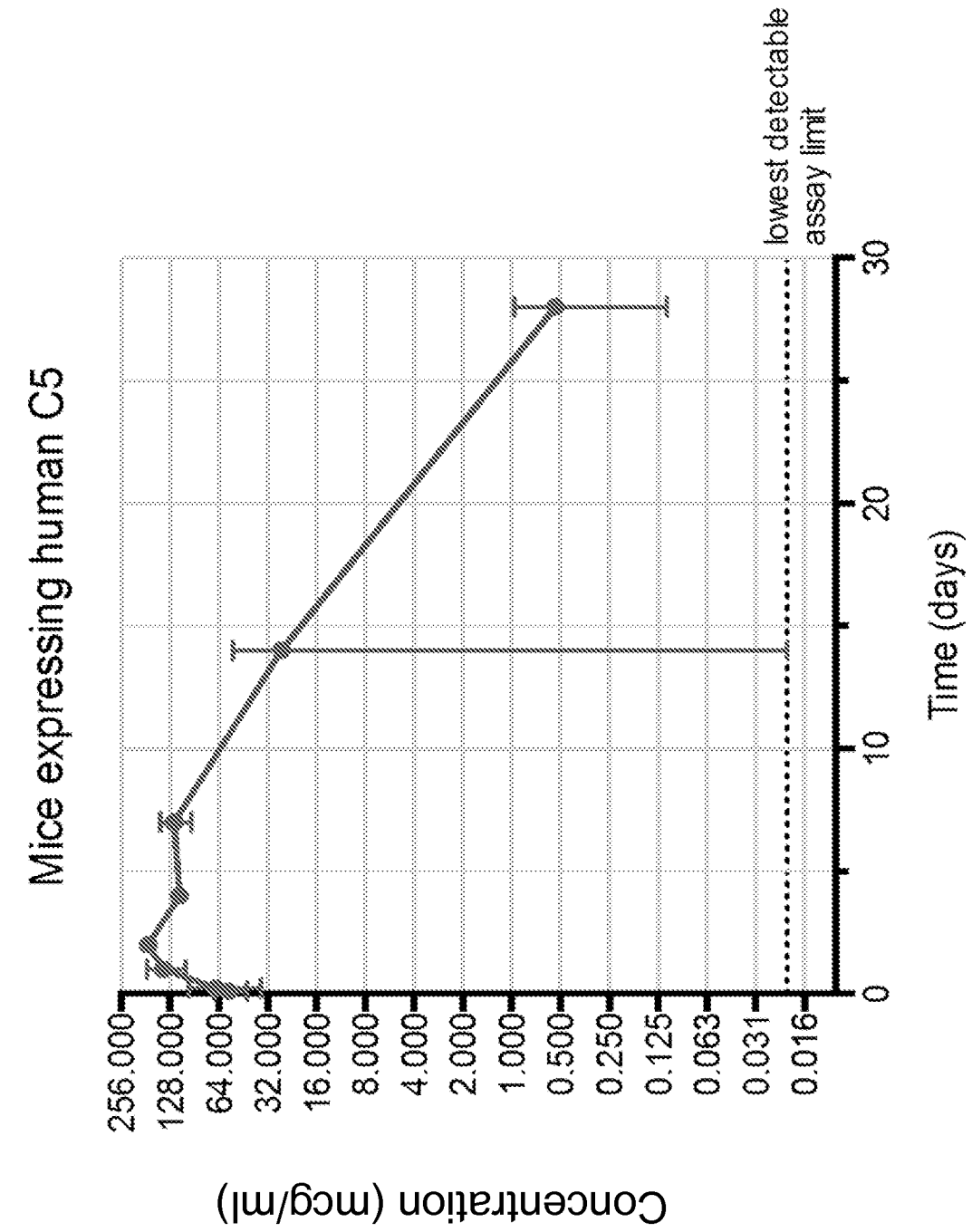

Mice expressing human C5
Concentration (mcg/ml)
256.000
128.000
64.000
32.000
16.000
8.000
4.000
2.000
1.000
0.500
0.250
0.125
0.063
0.031
0.016
lowest detectable assay limit
0
10
20
30
Time (days)

FIGURE 9A

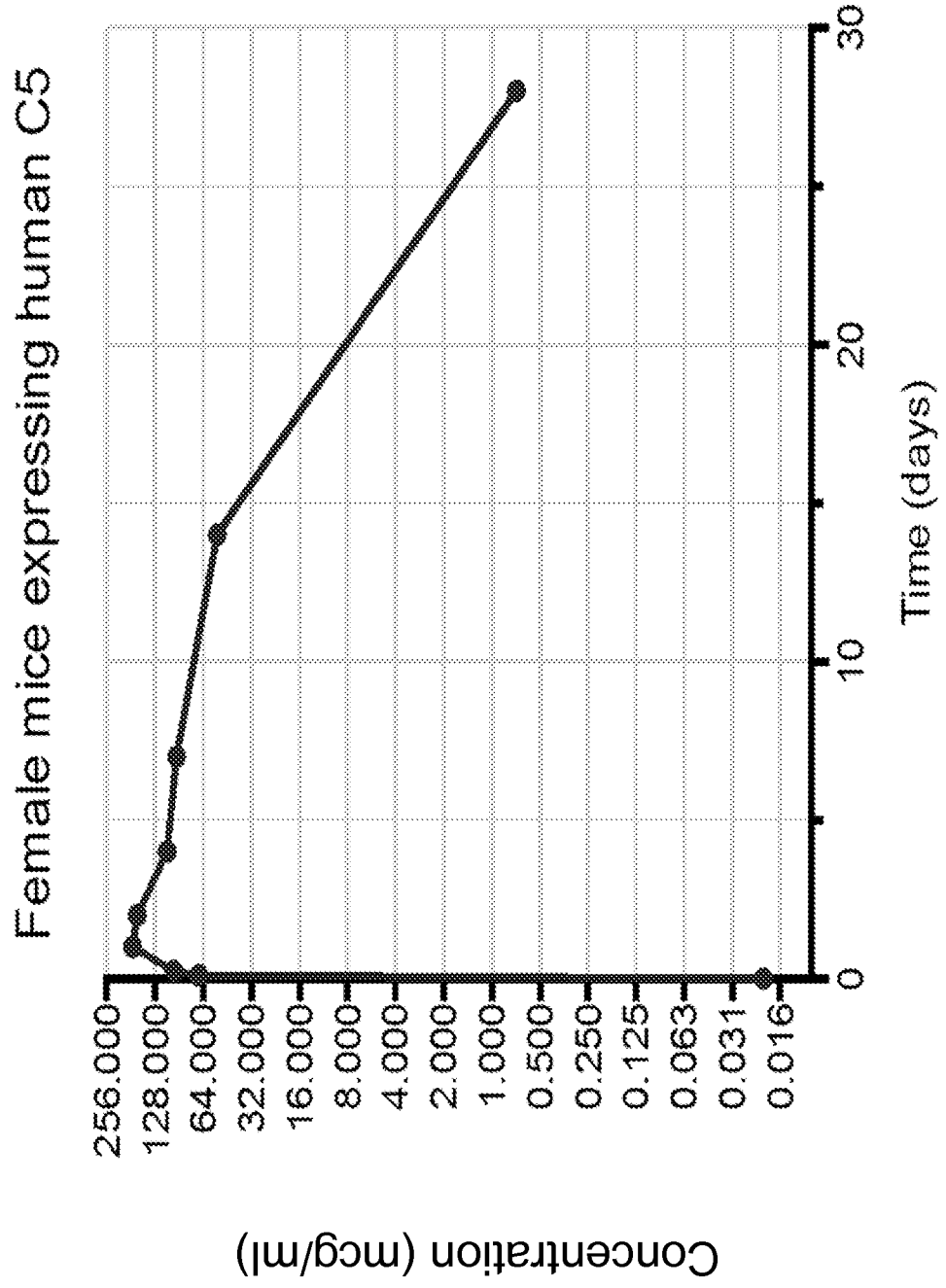

Female mice expressing human C5
Concentration (mcg/ml)
256.000
128.000
64.000
32.000
16.000
8.000
4.000
2.000
1.000
0.500
0.250
0.125
0.063
0.031
0.016
0
10
20
30
Time (days)

FIGURE 9B

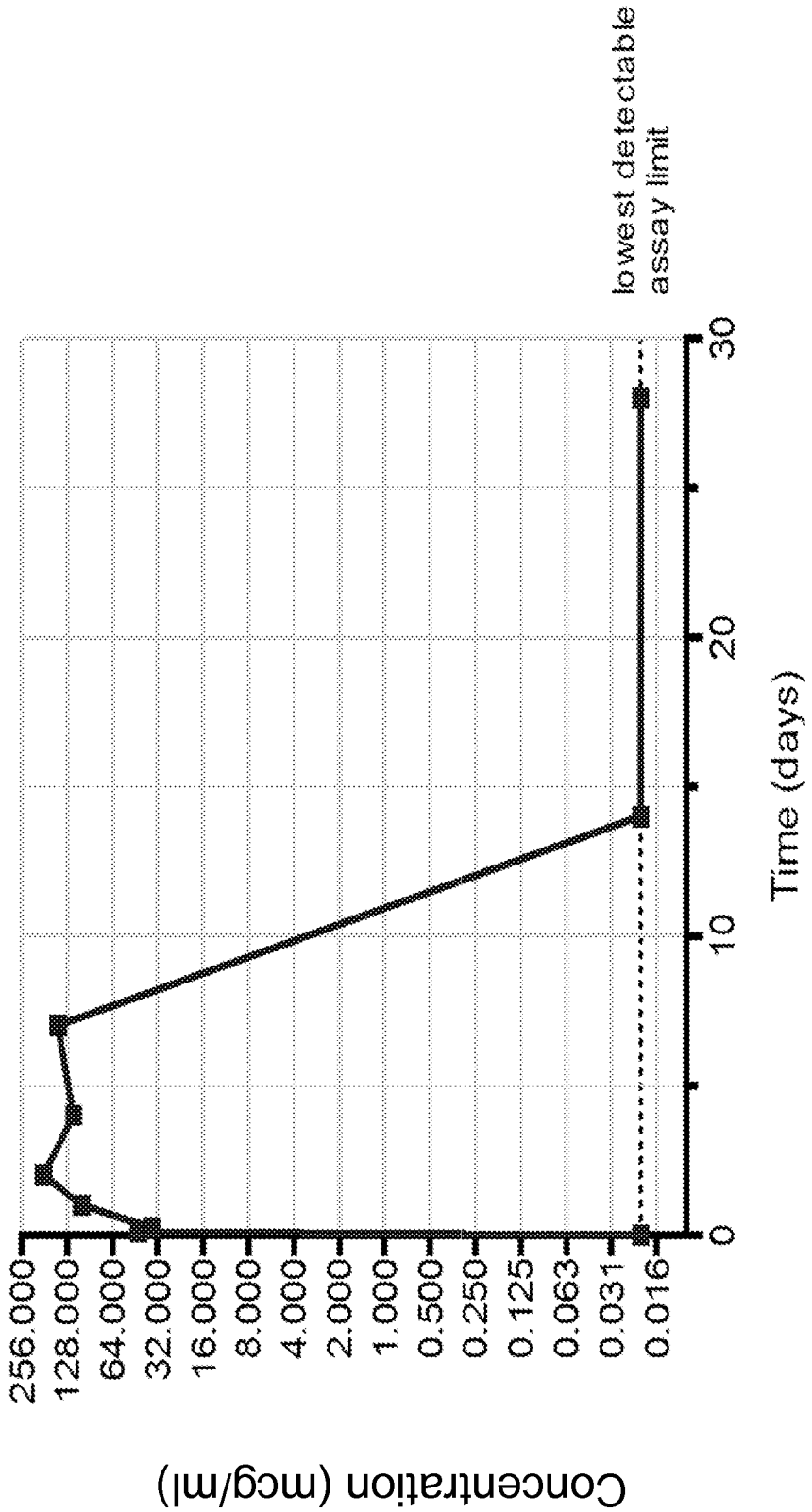


FIGURE 9B (CONT)

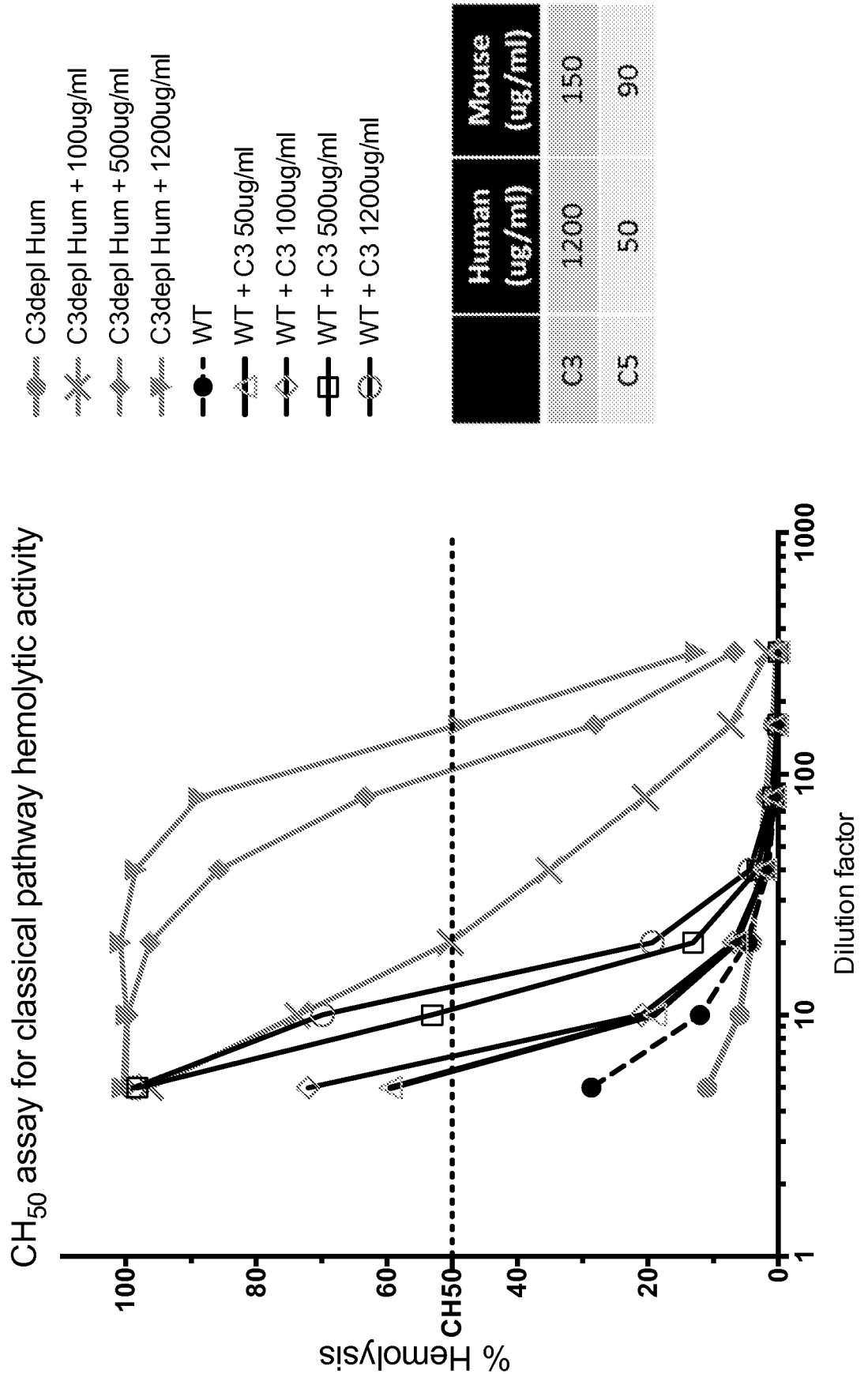

CH50 assay for classical pathway hemolytic activity
% Hemolysis
Dilution factor
CH50
0
20
40
60
80
100
1
10
100
1000
C3depl Hum
C3depl Hum + 100ug/ml
C3depl Hum + 500ug/ml
C3depl Hum + 1200ug/ml
WT
WT + C3 50ug/ml
WT + C3 100ug/ml
WT + C3 500ug/ml
WT + C3 1200ug/ml
Human (ug/ml)
Mouse (ug/ml)
C3
1200
150
C5
50
90

FIGURE 10A

	Human (ug/ml)	Mouse (ug/ml)
C3	1200	150
C5	50	90

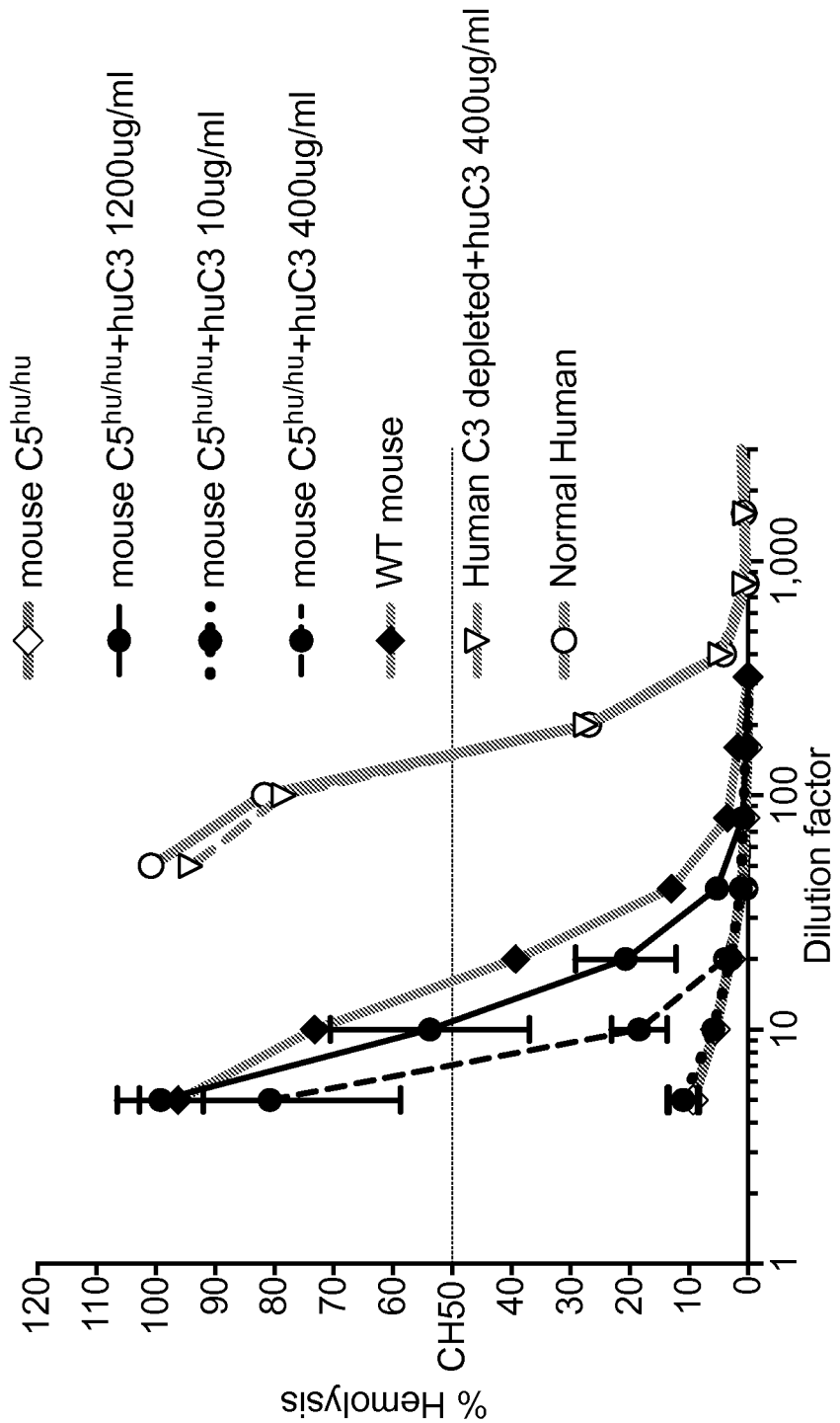
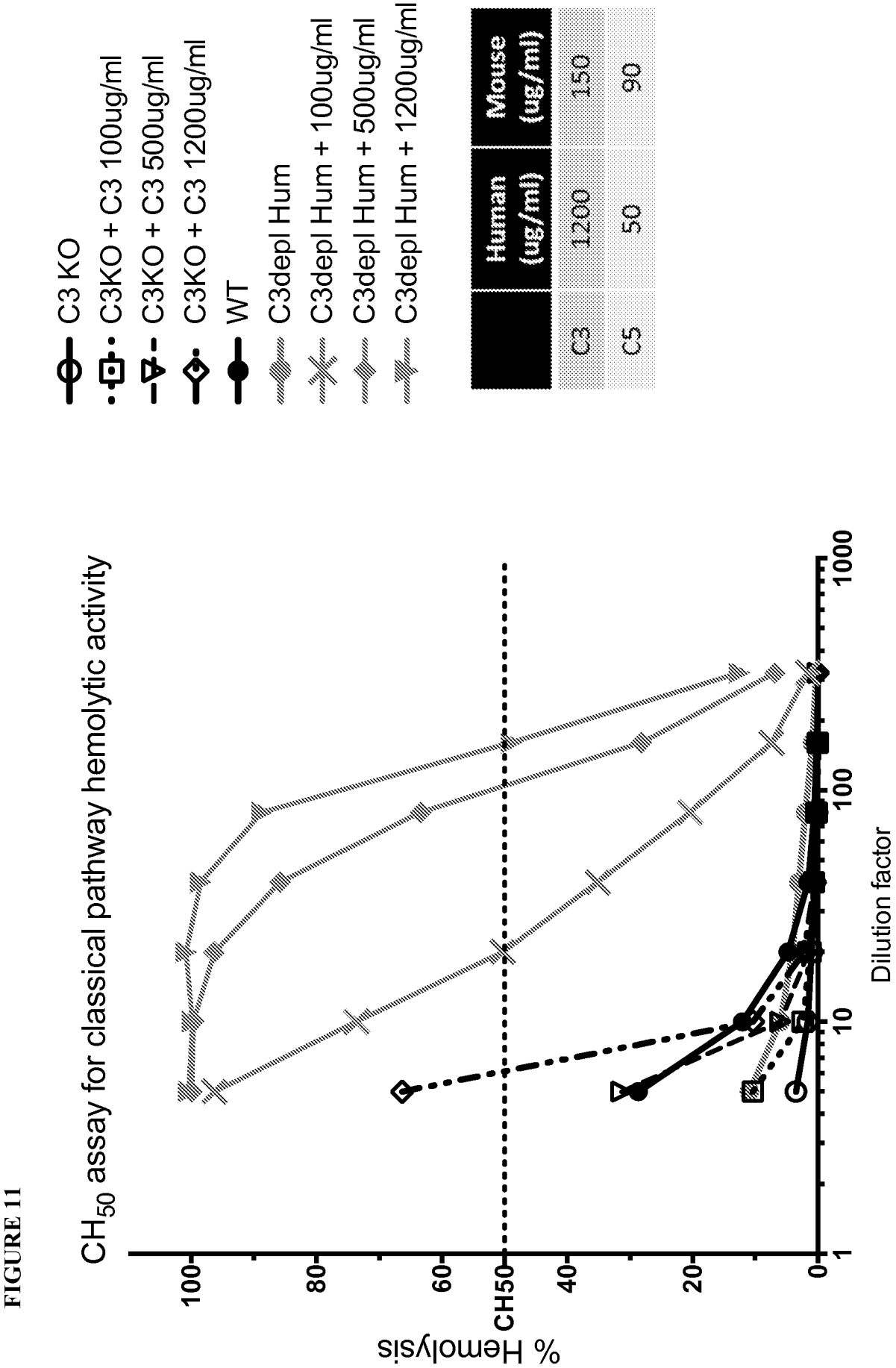


FIGURE 10B

FIGURE 11


CH50 assay for classical pathway hemolytic activity
% Hemolysis
CH50
Dilution factor
0
20
40
60
80
100
1
10
100
1000
C3 KO
C3KO + C3 100ug/ml
C3KO + C3 500ug/ml
C3KO + C3 1200ug/ml
WT
C3depl Hum
C3depl Hum + 100ug/ml
C3depl Hum + 500ug/ml
C3depl Hum + 1200ug/ml
Human (ug/ml)
Mouse (ug/ml)
C3
1200
150
C5
50
90

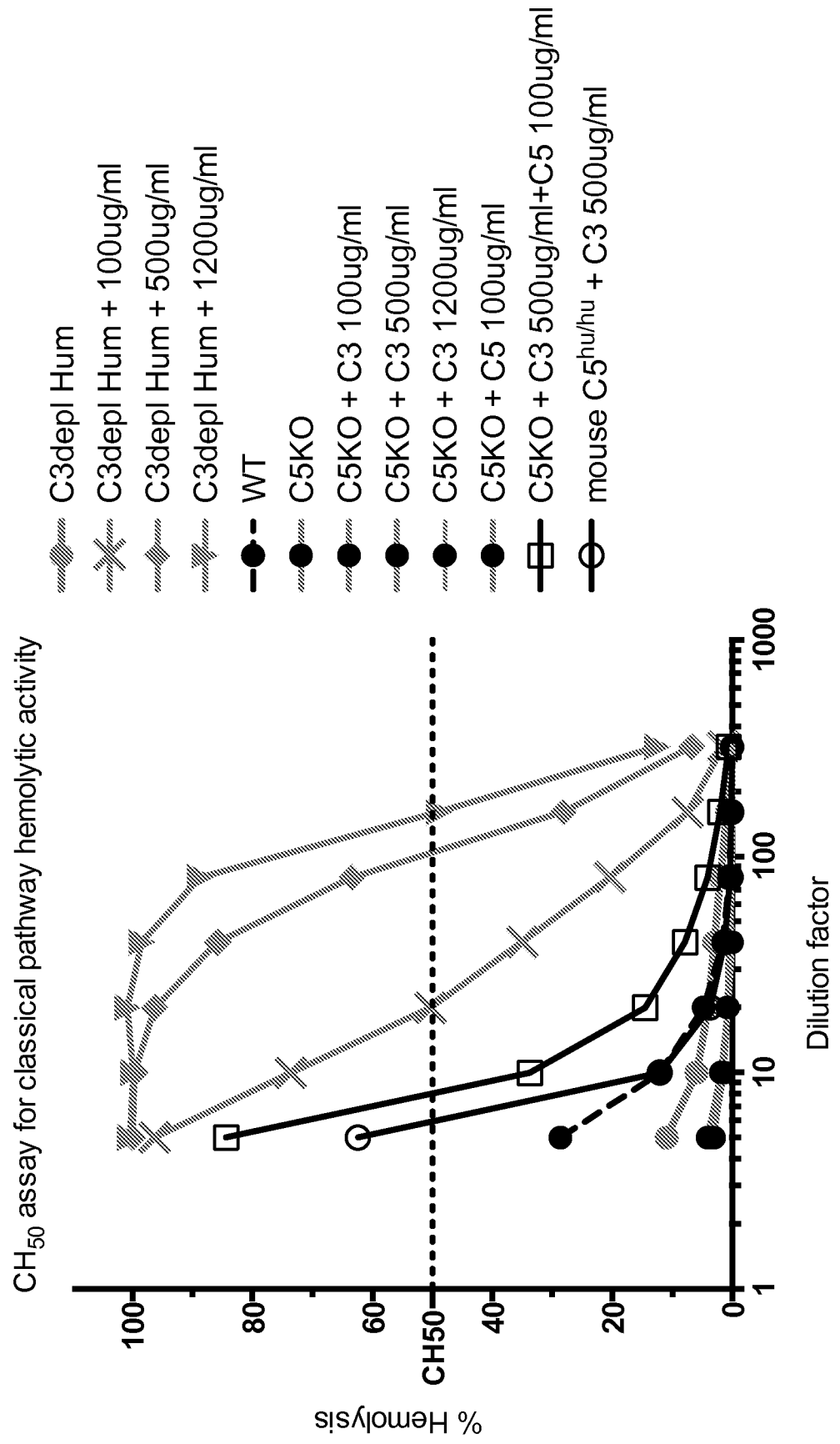

CH50 assay for classical pathway hemolytic activity
% Hemolysis
Dilution factor
CH50
0
20
40
60
80
100
1
10
100
1000
C3depl Hum
C3depl Hum + 100ug/ml
C3depl Hum + 500ug/ml
C3depl Hum + 1200ug/ml
WT
C5KO
C5KO + C3 100ug/ml
C5KO + C3 500ug/ml
C5KO + C3 1200ug/ml
C5KO + C5 100ug/ml
C5KO + C3 500ug/ml+C5 100ug/ml
mouse C5hu/hu + C3 500ug/ml

FIGURE 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/029111

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01K67/027 A61K49/00 C07K14/47 C12N15/85
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01K A61K C07K C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data, BIOSIS, Sequence Search, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ADRIANNA LATUSZEK ET AL: "Evaluation of complement activity in wild type, C5 knockout and humanized mice for drug discovery", 20140401 , vol. 55, no. 13 1 April 2014 (2014-04-01), XP007923220, Retrieved from the Internet: URL:http://iovs.arvojournals.org/article.aspx?articleid=2266543 the whole document -----	1-56
A	WO 2013/192030 A1 (REGENERON PHARMA [US]) 27 December 2013 (2013-12-27) -----	1-56
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 August 2015	28/08/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Puonti-Kaerlas, J

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/029111

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PHANOURIOS TAMAMIS ET AL: "Design of a modified mouse protein with ligand binding properties of its human analog by molecular dynamics simulations: The case of C3 inhibition by compstatin", PROTEINS: STRUCTURE, FUNCTION, AND BIOINFORMATICS, vol. 79, no. 11, 30 November 2011 (2011-11-30), pages 3166-3179, XP055209107, ISSN: 0887-3585, DOI: 10.1002/prot.23149 -----	1-56
A	WO 2005/060739 A1 (G2 INFLAMMATION PTY LTD [AU]; MACKAY CHARLES REAY [AU]) 7 July 2005 (2005-07-07) -----	1-56
A	RAFAEL L. UFRET-VINCENTY ET AL: "Transgenic Mice Expressing Variants of Complement Factor H Develop AMD-like Retinal Findings", INVESTIGATIVE OPTHALMOLOGY & VISUAL SCIENCE, vol. 51, no. 11, 1 November 2010 (2010-11-01), page 5878, XP055209111, ISSN: 1552-5783, DOI: 10.1167/iovs.09-4457 -----	1-56

]

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/029111

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013192030 A1	27-12-2013	AU 2013277457 A1	22-01-2015
		CA 2873134 A1	27-12-2013
		CN 104378976 A	25-02-2015
		EP 2861063 A1	22-04-2015
		IL 235773 A	29-01-2015
		KR 20150027752 A	12-03-2015
		US 2013340104 A1	19-12-2013
		US 2015082469 A1	19-03-2015
		WO 2013192030 A1	27-12-2013
WO 2005060739 A1	07-07-2005	AU 2004304665 A1	07-07-2005
		AU 2009201840 A1	04-06-2009
		EP 1701611 A1	20-09-2006
		EP 2322637 A1	18-05-2011
		US 2007277252 A1	29-11-2007
		WO 2005060739 A1	07-07-2005