AMENDED CLAIMS
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CLAIMS

What is claimed is:

1. A non-human animal comprising in its germline a genetically modified immunoglobulin heavy chain locus comprising an unrearranged human immunoglobulin heavy chain variable region nucleotide sequence, wherein the unrearranged heavy chain variable region nucleotide sequence comprises at least one histidine codon that is not encoded by a corresponding human germline heavy chain variable gene segment.

2. The non-human animal of claim 1, wherein the non-human animal is a mammal.

3. The non-human animal of claim 2, wherein the mammal is a rodent selected from the group consisting of a mouse, a rat, and a hamster.

4. The non-human animal of claim 1, wherein the added or substituted histidine codon is present in a human immunoglobulin heavy chain gene segment selected from a human V_H gene segment, a human D gene segment, a human J_H gene segment, and a combination thereof.

5. The non-human animal of claim 4, wherein the human immunoglobulin heavy chain gene segment is selected from a human germline V_H gene segment, a human germline D gene segment, a human germline J_H gene segment, and a combination thereof.

6. The non-human animal of claim 5, wherein the added or substituted histidine codon is present in at least one reading frame of the human immunoglobulin heavy chain gene segment that encodes a heavy chain variable domain selected from an N-terminal region, a loop 4 region, a complementary determining region 1 (CDR1), a complementary determining region 2 (CDR2), a complementary determining region 3 (CDR3), and a combination thereof.

7. The non-human animal of claim 1, wherein the endogenous non-histidine codon that is substituted with the histidine codon encodes the amino acid selected from the group consisting of Y, N, D, Q, S, W, and R.

8. The non-human animal of claim 1, wherein the added or substituted histidine codon is present in at least one reading frame of a human D gene segment.

9. The non-human animal of claim 8, wherein the reading frame is a hydrophilic frame of the human D gene segment, and the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, and a combination thereof.

10. The non-human animal of claim 1, wherein the unrearranged heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence.

11. The non-human animal of claim 10, wherein the unrearranged heavy chain variable region nucleotide sequence is operably linked to an endogenous non-human heavy chain constant region nucleotide sequence selected from the group consisting of a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof.

12. The non-human animal of claim 10, wherein the human heavy chain constant region nucleotide sequence comprises a modification that increases an affinity of a heavy chain constant region amino acid sequence to neonatal Fc receptor (FcRn) at a pH ranging from about 5.5 to about 6.0, wherein the modification is a mutation selected from the group consisting of M428L, N434S, M428L, V259I, V308F, N434A, M252Y, S254T, T256E T250Q, M248L, H433K, N434Y, and a combination thereof.

13. The non-human animal of claim 1, wherein the non-human animal is homozygous for the genetically modified immunoglobulin heavy chain locus in the germline.

14. The non-human animal of claim 1, wherein the non-human animal further comprises an unrearranged human immunoglobulin light chain V gene segment and an unrearranged human immunoglobulin light chain J gene segment.

15. The non-human animal of claim 1, wherein the non-human animal comprises an enriched B cell population that is capable of producing an antigen-binding protein comprising a heavy chain variable domain having at least one histidine residue.

16. The non-human animal of claim 15, wherein the B cell population has been activated with an antigen, and the antigen-binding protein has undergone affinity maturation through somatic hypermutation (SHM).

17. The non-human animal of claim 15, wherein the antigen-binding protein produced by the B cell population exhibits a decreased antigen-binding affinity at a pH ranging from about 5.5 to about 6.0 as compared with at a neutral pH ranging from about 7.0 to about 7.4.

18. The non-human animal of claim 1, wherein the non-human animal is heterozygous for the genetically modified immunoglobulin heavy chain locus in the germline, and the non-human animal comprises a B cell population that is capable of producing an antigen-binding protein comprising a heavy chain variable domain having at least one histidine residue.

19. The non-human animal of claim 1, wherein the non-human animal comprises an Adam6a gene, an Adam6b gene, or both.

20. A genetically modified immunoglobulin heavy chain locus in a germline of a non-human animal comprising an unrearranged human heavy chain variable region nucleotide sequence, wherein the unrearranged human heavy chain variable region nucleotide sequence comprises at least one histidine codon that is not encoded by a corresponding human germline heavy chain variable gene segment.

21. The genetically modified immunoglobulin heavy chain locus of claim 20, wherein the added or substituted histidine codon is present in an immunoglobulin heavy chain gene segment selected from a human V_H gene segment, a human D gene segment, a human J_H gene segment, and a combination thereof.

22. The genetically modified immunoglobulin heavy chain locus of claim 21, wherein the human immunoglobulin heavy chain gene segment is selected from a human germline V_H gene segment, a human germline D gene segment, a human

germline J_H gene segment, and a combination thereof.

23. The genetically modified immunoglobulin heavy chain locus of claim 20, wherein the added or substituted histidine codon is present in at least one reading frame encoding a heavy chain variable domain selected from an N-terminal region, a loop 4 region, a complementary determining region 1 (CDR1), a complementary determining region 2 (CDR2), a complementary determining region 3 (CDR3), and a combination thereof.

24. The genetically modified immunoglobulin heavy chain locus of claim 20, wherein the endogenous non-histidine codon that is replaced with the histidine codon encodes the amino acid selected from the group consisting of Y, N, D, Q, S, W, and R.

25. The genetically modified immunoglobulin heavy chain locus of claim 20, wherein the added or substituted histidine codon is present in at least one reading frame of a human D gene segment.

26. The genetically modified immunoglobulin heavy chain locus of claim 25, wherein the reading frame is a hydrophilic frame of the human D gene segment, and the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, and a combination thereof.

27. The genetically modified immunoglobulin heavy chain locus of claim 20, wherein the unrearranged heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from the group consisting of a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof.

28. The genetically modified immunoglobulin heavy chain locus of claim 27, wherein the unrearranged heavy chain variable region nucleotide sequence is operably linked to an endogenous non-human heavy chain constant region

nucleotide sequence selected from the group consisting of a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof.

29. The genetically modified immunoglobulin locus of claim 27, wherein the human heavy chain constant region nucleotide sequence comprises a modification that increases an affinity of a heavy chain constant region amino acid sequence to neonatal Fc receptor (FcRn) at a pH ranging from about 5.5 to about 6.0, wherein the modification is a mutation selected from the group consisting of M428L, N434S, M428L, V259I, V308F, N434A, M252Y, S254T, T256E T250Q, M248L, H433K, N434Y, and a combination thereof.

30. A method of making a non-human animal that comprises a genetically modified immunoglobulin heavy chain locus in its germline genome, the method comprising:

(a) modifying a genome of a non-human animal to delete or render non-functional endogenous immunoglobulin heavy chain V, D, and J gene segments in an immunoglobulin heavy chain locus; and

(b) placing in the genome an unrearranged human heavy chain variable region nucleotide sequence comprising an addition of at least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon.

31. The method of claim 30, wherein the added or substituted histidine codon is present in an immunoglobulin heavy chain gene segment selected from a human V_H gene segment, a human D gene segment, a human J_H gene segment, and a combination thereof.

32. The method of claim 31, wherein the human immunoglobulin heavy chain gene segment is selected from a human germline V_H gene segment, a human germline D gene segment, a human germline J_H gene segment, and a combination thereof.

33. The method of claim 30, wherein the added or substituted histidine codon is present in at least one reading frame encoding a heavy chain variable domain selected from an N-terminal region, a loop 4 region, a complementary determining region 1 (CDR1), a complementary determining region 2 (CDR2), a complementary

determining region 3 (CDR3), and a combination thereof.

34. The method of claim 30, wherein the endogenous non-histidine codon that is replaced with the histidine codon encodes the amino acid selected from the group consisting of Y, N, D, Q, S, W, and R.

35. The method of claim 30, wherein the added or substituted histidine codon is present in one or more reading frame of a human D gene segment.

36. The method of claim 35, wherein the reading frame is a hydrophilic frame of the human D gene segment, and the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, and a combination thereof.

37. The method of claim 30, wherein the unrearranged heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from the group consisting of a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof.

38. The method of claim 37, wherein the unrearranged heavy chain variable region nucleotide sequence is operably linked to an endogenous non-human heavy chain constant region nucleotide sequence selected from the group consisting of a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof.

39. The method of claim 38, wherein the human heavy chain constant region nucleotide sequence comprises a modification that increases an affinity of a heavy chain constant region amino acid sequence to neonatal Fc receptor (FcRn) at a pH ranging from about 5.5 to about 6.0, wherein the modification is a mutation selected from the group consisting of M428L, N434S, M428L, V259I, V308F, N434A, M252Y, S254T, T256E T250Q, M248L, H433K, N434Y, and a combination thereof.

40. The method of claim 30, wherein the non-human animal is homozygous for the genetically modified immunoglobulin heavy chain locus in the germline genome.

41. The method of claim 30, wherein the non-human animal comprising the genetically modified immunoglobulin heavy chain locus comprises a B cell population that is capable of producing an antigen-binding protein comprising a heavy chain variable domain having at least one histidine residue.

42. The method of claim 41, wherein the B cell population has been activated with an antigen, and the antigen-binding protein has undergone affinity maturation through somatic hypermutation (SHM).

43. The method of claim 41, wherein the antigen-binding protein produced by the B cell population exhibits a decreased antigen-binding affinity at a pH ranging from about 5.5 to about 6.0 as compared with at a neutral pH ranging from about 7.0 to about 7.4.

44. The method of claim 29, wherein the non-human animal comprises an Adam6a gene, an Adam6b gene, or both.

45. The method of claim 29, wherein the method results in a genetically modified animal that comprises a population of B cells enriched for antibodies exhibiting pH-dependent binding to an antigen of interest.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A non-human animal comprising in its germline a genetically modified immunoglobulin heavy chain locus comprising an unrearranged human immunoglobulin heavy chain variable region nucleotide sequence, wherein the unrearranged heavy chain variable region nucleotide sequence comprises at least one histidine codon that is not encoded by a corresponding human germline heavy chain variable region gene segment, and wherein the histidine codon is present in a complementary determining region (CDR3) encoding sequence.

2. The non-human animal of claim 1, wherein the non-human animal is a mammal, optionally wherein the mammal is a rodent selected from the group consisting of a mouse, a rat, and a hamster.

3. The non-human animal of claim 1 or 2, wherein the histidine codon is present in a human immunoglobulin heavy chain gene segment selected from a human V_H gene segment, a human D gene segment, a human J_H gene segment, and a combination thereof.

4. The non-human animal of claim 3, wherein the human immunoglobulin heavy chain gene segment is selected from a human germline V_H gene segment, a human germline D gene segment, a human germline J_H gene segment, and a combination thereof.

5. The non-human animal of claim 1, wherein:

(I) an endogenous non-histidine codon encoding an amino acid selected from the group consisting of Y, N, D, Q, S, W, and R is substituted with said at least one histidine codon;

(II) the histidine codon is present in at least one reading frame of a human D gene segment, optionally

wherein the reading frame is a hydrophilic frame of the human D gene segment, and the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, and a combination thereof;

(III) the unrearranged heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence; optionally wherein - the unrearranged heavy chain variable region nucleotide sequence is operably linked to an endogenous non-human heavy chain constant region nucleotide sequence selected from the group consisting of a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof; or wherein - the human heavy chain constant region nucleotide sequence comprises a modification that increases an affinity of a CH_2-CH_3 region of IgG heavy chain constant region amino acid sequence to neonatal Fc receptor (FcRn) at a pH ranging from about 5.5 to about 6.0, wherein the modification is a mutation selected from the group consisting of M428L, N434S, V259I, V308F, N434A, M252Y, S254T, T256E T250Q, H433K, N434Y, and a combination thereof;

(IV) the non-human animal is homozygous for the genetically modified immunoglobulin heavy chain locus in the germline;

(V) the non-human animal further comprises an unrearranged human immunoglobulin light chain V gene segment and an unrearranged human immunoglobulin light chain J gene segment;

(VI) the non-human animal comprises an enriched B cell population that is capable of producing an antigen-binding protein comprising a heavy chain variable domain having at least one histidine residue, optionally

wherein - the B cell population has been activated with an antigen, and the antigen-binding protein has undergone affinity maturation through somatic hypermutation (SHM), or

wherein - the antigen-binding protein produced by the B cell population exhibits a decreased antigen-binding affinity at a pH ranging from about 5.5 to about 6.0 as compared with at a neutral pH ranging from about 7.0 to about 7.4; or

(VII) the non-human animal is heterozygous for the genetically modified immunoglobulin heavy chain locus in the germline, and the non-human animal comprises a B cell population that is capable of producing an antigen-binding protein comprising a heavy chain variable domain having at least one histidine residue; or

(VIII) the non-human animal comprises an Adam6a gene, an Adam6b gene, or both.

6. A method of making a non-human animal that comprises a genetically modified immunoglobulin heavy chain locus in its germline genome, the method comprising:

(a) modifying a genome of a non-human animal to delete or render non-functional endogenous immunoglobulin heavy chain V, D, and J gene segments in an immunoglobulin heavy chain locus; and

(b) placing in the genome an unrearranged human heavy chain variable region nucleotide sequence comprising an addition of at least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon, wherein the histidine codon is present in a complementary determining region (CDR3) encoding sequence.

7. The method of claim 6, wherein the added or substituted histidine codon is present in an immunoglobulin heavy chain gene segment selected from a human V_H gene segment, a human D gene segment, a human J_H gene segment, and a combination thereof.

8. The method of claim 7, wherein the human immunoglobulin heavy chain gene segment is selected from a human germline V_H gene segment, a human germline D gene segment, a human germline J_H gene segment, and a combination thereof.

9. The method of claim 6, wherein:

(I) the endogenous non-histidine codon that is replaced with the histidine codon encodes the amino acid selected from the group consisting of Y, N, D, Q, S, W, and R; or

(II) the added or substituted histidine codon is present in one or more reading frame of a human D gene segment, optionally

wherein the reading frame is a hydrophilic frame of the human D gene segment, and the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, and a combination thereof.

10. The method of claim 6, wherein the unrearranged heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant

region nucleotide sequence selected from the group consisting of a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof.

11. The method of claim 10, wherein:

(I) the unrearranged heavy chain variable region nucleotide sequence is operably linked to an endogenous non-human heavy chain constant region nucleotide sequence selected from the group consisting of a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof, optionally

wherein the human heavy chain constant region nucleotide sequence comprises a modification that increases an affinity of a CH_2-CH_3 region of an IgG heavy chain constant region amino acid sequence to neonatal Fc receptor (FcRn) at a pH ranging from about 5.5 to about 6.0, wherein the modification is a mutation selected from the group consisting of M428L, N434S, V259I, V308F, N434A, M252Y, S254T, T256E T250Q, H433K, N434Y, and a combination thereof;

(II) the non-human animal is homozygous for the genetically modified immunoglobulin heavy chain locus in the germline genome;

(III) the non-human animal comprises an Adam6a gene, an Adam6b gene, or both; or

(IV) the method results in a genetically modified animal that comprises a population of B cells enriched for antibodies exhibiting pH-dependent binding to an antigen of interest.

12. The method of claim 6, wherein the non-human animal comprising the genetically modified immunoglobulin heavy chain locus comprises a B cell population that is capable of producing an antigen-binding protein comprising a heavy chain variable domain having at least one histidine residue.

13. The method of claim 12, wherein:

(I) the B cell population has been activated with an antigen, and the antigen-binding protein has undergone affinity maturation through somatic hypermutation (SHM); or

(II) the antigen-binding protein produced by the B cell population exhibits a decreased antigen-binding affinity at a pH ranging from about 5.5 to about 6.0 as compared with at a neutral pH ranging from about 7.0 to about 7.4.

14. A method for obtaining an antigen-binding protein with enhanced recyclability and/or improved serum half-life, comprising:

(a) Immunising a non-human animal comprising in its germline a genetically modified immunoglobulin heavy chain locus comprising an unrearranged human immunoglobulin heavy chain variable region nucleotide sequence, wherein the unrearranged heavy chain variable region nucleotide sequence comprises at least one histidine codon that is not encoded by a corresponding human germline heavy chain variable region gene segment, and wherein the histidine codon is present in a complementary determining region (CDR3) encoding sequence;

(b) allowing the non-human animal to mount an immune response;

(c) harvesting a lymphocyte (*e.g.*, a B cell) from the immunized non-human animal;

(d) fusing the lymphocyte with a myeloma cell to form a hybridoma cell, and

(e) obtaining an antigen-binding protein produced by the hybridoma cell, wherein the antigen-binding protein exhibits enhanced recyclability and/or serum stability.

15. The method of claim 14, wherein said non-human animal is as defined in any one of claims 2 to 5.