Mice That Produce Antigen-Binding Proteins With pH-Dependent Binding Characteristics

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Application No. 61/611,950, filed 16 March 2012, U.S. Provisional Application No. 61/613,352, filed March 20 2012, and U.S. Provisional Application No. 61/736,930, filed 13 December 2012, the entire contents of each of the applications are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] Genetically modified immunoglobulin loci of non-human animals comprising an unrearranged human heavy chain variable region nucleotide sequence, wherein the unrearranged human heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one non-histidine codon with a histidine codon. Non-human animals, including rodents, *e.g.,* mice and rats, comprising in their germline an unrearranged human immunoglobulin heavy chain variable region nucleotide sequence, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one non-histidine codon with a histidine codon. Genetically engineered non-human animals capable of expressing an antigen-binding protein that is characterized by pH-dependent antigen binding, improved recyclability, and/or enhanced serum half-life.

BACKGROUND OF THE INVENTION

[0003] Drugs administered into the body, including therapeutic monoclonal antibodies, can be affected via various elimination mechanisms, including glomerular filtration (*e.g.*, into urine), secretion (*e.g.*, into the bile), and catabolism by cells. While small molecules are cleared from the body via renal filtration, the majority of secreted antibodies (*e.g.*, IgG, which are too big to be filtered through glomeruli) are primarily removed from the body via cell-mediated catabolism, *e.g.*, fluid-phase endocytosis (phagocytosis) or receptor-mediated endocytosis. For example, soluble molecules with several repeated epitopes are bound by a plurality of circulating antibodies, and the resulting large antigen-antibody complexes are phagocytosed rapidly into cells for degradation. On the other hand, cell surface target receptors, which are bound by antibodies (*i.e.*, receptor-antibody complexes), undergo target-mediated endocytosis in a dose-dependent manner, which leads to formation of endosomes destined for lysosomal degradation inside cells. In some cases, the endocytosed receptor-antibody complexes bind neonatal Fc receptors (FcRn) inside the

endosomes in a pH-dependent manner and are routed back to the cell surface for release into plasma or interstitial fluids upon exposure to a neutral extracellular pH (*e.g.,* pH 7.0-7.4).

[0004] There is a need in the art for systems, *e.g.,* non-human animals, cells, and genomic loci that generate antigen-binding proteins with titratable residues, *e.g.,* genetically modified loci that rearrange immunoglobulin gene segments to generate heavy chain variable domains that respond to changes in pH, *e.g.,* that donate or accept protons and, *e.g.,* whose binding characteristics differ according to protonation state.

[0005] There is also a need in the art for methods and compositions that can further increase recycling efficiency of endocytosed antigen-binding proteins by promoting dissociation of antigen-binding proteins from receptor-antigen-binding protein complexes or by increasing the affinity of antigen-binding proteins toward FcRn in an acidic endosomal compartment without compromising the specificity and affinity of the antigen-binding protein toward an antigen of interest.

SUMMARY OF THE INVENTION

[0006] Genetically modified immunoglobulin heavy chain loci in the germline genome of non-human animals are provided, wherein the immunoglobulin heavy chain loci comprise a genetically modified unrearranged heavy chain variable region nucleotide sequence (*e.g.*, one or more genetically modified human V_H, D, and/or J_H gene segment), wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of at least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon. In various embodiments, the genetically modified unrearranged heavy chain variable region nucleotide sequence comprises at least one histidine codon in at least one reading frame that encodes an immunoglobulin heavy chain variable domain. In various embodiments, the unrearranged heavy chain variable region nucleotide sequence comprising the at least one histidine codon is operably linked to a human or non-human heavy chain constant region nucleotide sequence (*e.g.*, a heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgA, IgE, and IgG).

[0007] Non-human animals (mammals, *e.g.*, rodents such as mice, rats, or hamsters) are provided that are genetically engineered to contain immunoglobulin heavy chain genomic loci in their germline genome, wherein the genomic loci comprise an unrearranged heavy chain variable region nucleotide sequence (*e.g.*, one or more genetically modified human V_H, D, and/or J_H gene segments), wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of at least one histidine codon or a

substitution of at least one endogenous non-histidine codon with a histidine codon. In various embodiments, the genome of the non-human animals comprises a modification (i) that deletes or renders nonfunctional all, or substantially all, endogenous immunoglobulin V_H, D, and/or J_H gene segments (*e.g.*, via insertion of a nucleotide sequence, *e.g.*, an exogenous nucleotide sequence, in the immunoglobulin locus or via non-functional rearrangement or inversion of endogenous V_H, D, and/or J_H gene segments); and (ii) that introduces an unrearranged human heavy chain variable region nucleotide sequence (*e.g.*, genetically modified human V_H, D, or J_H gene segments), wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of at least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon. In various embodiments, the unrearranged heavy chain variable region nucleotide sequence is present at an endogenous locus (*i.e.*, where the unrearranged heavy chain variable region nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.*, at a locus different from the endogenous immunoglobulin heavy chain locus in its genome), or within its endogenous locus (*e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome). In various embodiments, the immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence (*e.g.*, a heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgA, IgE, and IgG).

[0008] Genetically modified non-human animals are provided that are capable of expressing a genetically modified immunoglobulin heavy variable domain comprising one or more histidines, wherein the one or more histidines are not encoded by a germline gene segment of a corresponding wild-type non-human animal.

[0009] Genetically modified non-human animals are provided that comprise a B cell population that is characterized by rearranged immunoglobulin heavy chain variable genes that encode an immunoglobulin heavy chain variable domain with one or more histidines that are not encoded by a germline gene segment of a corresponding wild-type non-human animal.

[00010] Methods and compositions are provided for making non-human animals that comprise a genetically modified immunoglobulin heavy chain variable locus comprising an unrearranged human heavy chain variable region nucleotide sequence containing one or more histidine codons in at least one reading frame that encodes a heavy chain variable domain.

[00011] Methods and compositions are provided for non-human animals that make antigen-binding proteins that exhibit a pH-dependent binding of an antigen. Methods and compositions are provided for making non-human animals that have B cell populations, or antibody populations, that are enriched (as compared with corresponding wild-type animals) with antigen-binding proteins that are pH-dependent, *e.g.,* in particular, heavy chain variable domains, and/or antigen-binding fragments thereof.

[00012] In one aspect, a genetically modified immunoglobulin locus in a germline genome of a non-human animal is provided comprising an unrearranged human heavy chain variable region nucleotide sequence, wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon.

[00013] In one embodiment, the non-human animal is a mammal, including a rodent, *e.g.,* a mouse, a rat, or a hamster.

[00014] In one embodiment, 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. In one embodiment, the 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.

[00015] In one embodiment, the human V gene segment (V_H) is selected from the group consisting of V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23, V_H3-30, V_H3-30-3, V_H 3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, V_H7-81, and a combination thereof.

[00016] In one embodiment, the human D gene segment is selected from the group consisting of D1-1, D1-7, D1-14, D1-20, D1-26, D2-2, D2-8, D2-15, D2-21, D3-3, D3-9, D3-10, D3-16, D3-22, D4-4, D4-11, D4-17, D4-23, D5-12, D5-5, D5-18, D5-24, D6-6, D6-13, D6-19, D6-25, D7-27, and a combination thereof.

[00017] In one embodiment, the human J gene segment is selected from the group consisting of J_H1, J_H2, J_H3, J_H4, J_H5, J_H6, and a combination thereof.

[00018] In one embodiment, the added or substituted histidine codon is present in the unrearranged heavy chain variable region nucleotide sequence that encodes an N-terminal region, a loop 4 region, a CDR1, a CDR2, a CDR3, or a combination thereof.

[00019] In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprises 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, or 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, 40 or more, 41 or more, 42 or more, 43 or more, 44 or more, 45 or more, 46 or more, 47 or more, 48 or more, 49 or more, 50 or more, 51 or more, 52 or more, 53 or more, 54 or more, 55 or more, 56 or more, 57 or more, 58 or more, 59 or more, 60 or more, or 61 or more of histidine codons.

[00020] In one embodiment, the unrearranged heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

[00021] In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (*i.e.*, C_H1-hinge-C_H2-C_H3).

[00022] In one embodiment, a heavy chain constant region nucleotide sequence is present at an endogenous locus (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.*, at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

[00023] In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[00024] In one embodiment, the heavy chain constant region nucleotide sequence

encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (*e.g.,* E or Q); 250 and 428 (*e.g.,* L or F); 252 (*e.g.,* L/Y/F/W or T), 254 (*e.g.,* S or T), and 256 (*e.g.,* S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.,* L/R/S/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.*, in an endosome where pH ranges from about 5.5 to about 6.0).

[00025] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[00026] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[00027] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[00028] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

[00029] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

[00030] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

[00031] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

[00032] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

[00033] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

[00034] In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1, which is incorporated by reference herein in its entirety).

[00035] In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon numbering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

[00036] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and

V422I by EU).

[00037] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

[00038] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

[00039] In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[00040] In one embodiment, all or substantially all endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (*e.g.*, via insertion of a nucleotide sequence (*e.g.*, an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, *e.g.*, about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, *e.g.,* at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

[00041] In one embodiment, the genetically modified immunoglobulin heavy chain locus comprises a modification that deletes or renders non-functional all, or substantially all, endogenous V_H, D, and J_H gene segments; and the genetically modified locus comprises an unrearranged heavy chain variable region nucleotide sequence comprising one or more human V_H, D, and/or J_H gene segments having one or more histidine codons, wherein the unrearranged heavy chain variable region nucleotide sequence is present at an endogenous location (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.,* at a locus different from the endogenous immunoglobulin chain locus in its genome), or within its endogenous locus (*e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

[00042] In one embodiment, the genetically modified immunoglobulin locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

[00043] In one embodiment, the genetically modified immunoglobulin locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a human Adam6b gene.

[00044] In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized, unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

[00045] In one embodiment, the genetically modified immunoglobulin locus comprises an unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse κ locus. In one embodiment, the mouse immunoglobulin light chain locus is

a mouse λ locus.

[00046] In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglobulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

[00047] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (*e.g.,* at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

[00048] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (*e.g.,* pH of about 7.0 to about 7.4).

[00049] In one embodiment, the genetically modified immunoglobulin locus described herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, *e.g.*, antibodies, comprising a heavy chain

variable domain with one or more histidine residues. The antigen-binding proteins as described herein, when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

[00050] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification as described herein.

[00051] In one aspect, a genetically modified immunoglobulin locus in a germline genome of a non-human animal is provided comprising an unrearranged human heavy chain variable region nucleotide sequence, wherein the human unrearranged heavy chain variable region nucleotide sequence comprises a substitution of at least one endogenous non-histidine codon with a histidine codon.

[00052] In one embodiment, the non-human animal is a mammal, including a rodent, *e.g.,* a mouse, a rat, or a hamster.

[00053] In one embodiment, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, 40 or more, 41 or more, 42 or more, 43 or more, 44 or more, 45 or more, 46 or more, 47 or more, 48 or more, 49 or more, 50 or more, 51 or more, 52 or more, 53 or more, 54 or more, 55 or more, 56 or more, 57 or more, 58 or more, 59 or more, 60 or more, or 61 or more of the endogenous non-histidine codons are replaced with histidine codons.

[00054] In one embodiment, the endogenous non-histone codon encodes the amino acid selected from Y, N, D, Q, S, W, and R.

[00055] In one embodiment, the substituted histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes an immunoglobulin variable domain selected from an N-terminal region, a loop 4 region, a CDR1, a CDR2, a CDR3, a combination thereof.

[00056] In one embodiment, the substituted histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes a complementary determining region (CDR) selected from a CDR1, a CDR2, a CDR3, and a combination thereof.

[00057] In one embodiment, the substituted histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes a frame region (FR) selected from FR1, FR2, FR3, FR4, and a combination thereof.

[00058] In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprises a genetically modified human V_H gene segment, wherein one or more endogenous non-histidine codon in at least one reading frame of the human V_H gene segment has been replaced with a histidine codon.

[00059] In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a modification that replaces at least one endogenous non-histidine codon of a human V_H gene segment with a histidine codon, wherein the human V_H gene segment is selected from the group consisting of V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23, V_H3-30, V_H3-30-3, V_H 3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, V_H7-81, and a combination thereof.

[00060] In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a genetically modified human J_H gene segment, wherein one or more endogenous non-histidine codon in at least one reading frame of the human J_H gene segment has been replaced with a histidine codon.

[00061] In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a modification that replaces at least one endogenous non-

histidine codon of a human J_H segment with a histidine codon, wherein the human J_H gene segment is selected from the group consisting of J_H1, J_H2, J_H3, J_H4, J_H5, J_H6, and a combination thereof.

[00062] In one embodiment, the substituted histidine codon is present in a heavy chain variable region nucleotide sequence that encodes part of a CDR3. In one embodiment, the part of CDR3 comprises an amino acid sequence derived from a reading frame of a genetically modified human D gene segment comprising a modification that replaces at least one endogenous non-histidine codon in the reading frame with a histidine codon.

[00063] In one embodiment, the endogenous non-histidine codon that is substituted with a histidine codon encodes the amino acid selected from Y, N, D, Q, S, W, and R.

[00064] In one embodiment, the substituted histidine codon is present in at least one reading frame of the human D gene segment that is most frequently observed in VELOCIMMUNE® humanized immunoglobulin mice.

[00065] In one embodiment, the reading frame of the genetically modified human D gene segment that encodes part of CDR3 is selected from a hydrophobic frame, a stop frame, and a hydrophilic frame.

[00066] In one embodiment, the reading frame is a hydrophobic frame of a human D gene segment.

[00067] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (GTTGT; SEQ ID NO: 88), D1-7 (GITGT; SEQ ID NO: 89), D1-20 (GITGT; SEQ ID NO: 89), and D1-26 (GIVGAT; SEQ ID NO: 90), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[00068] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (DIVVVPAAI; SEQ ID NO: 92), D2-8 (DIVLMVYAI; SEQ ID NO: 94), D2-15 (DIVVVVAAT; SEQ ID NO: 95), and D2-21 (HIVVVTAI; SEQ ID NO: 97), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[00069] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the

group consisting of D3-3 (ITIFGVVII; SEQ ID NO: 98), D3-9 (ITIF*LVII; SEQ ID NO: 99, SEQ ID NO:100), D3-10 (ITMVRGVII; SEQ ID NO:101), D3-16 (IMITFGGVIVI; SEQ ID NO:102), and D3-22 (ITMIVVVIT; SEQ ID NO:103), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon.

[00070] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (TTVT; SEQ ID NO: 105), D4-11 (TTVT; SEQ ID NO:105), D4-17 (TTVT; SEQ ID NO:105), D4-23 (TTVVT; SEQ ID NO: 106) and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[00071] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (VDTAMV; SEQ ID NO: 107), D5-12 (VDIVATI; SEQ ID NO: 108), D5-18 (VDTAMV; SEQ ID NO:107), and D5-24 (VEMATI; SEQ ID NO:109), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[00072] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (SIAAR; SEQ ID NO: 111), D6-13 (GIAAAG; SEQ ID NO: 113), and D6-19 (GIAVAG; SEQ ID NO:115), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[00073] In one embodiment, the hydrophobic frame comprises a nucleotide sequence that encodes human D7-27 (LTG), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[00074] In one embodiment, the reading frame is a stop reading frame of a human D gene segment.

[00075] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (VQLER; SEQ ID NO:8), D1-7(V*LEL), D1-20(V*LER), D1-26 (V*WELL; SEQ ID NO: 12), and the human D gene segment further comprises a

modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[00076] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (RIL**YQLLY; SEQ ID NO:14), D2-8 (RILY*WCMLY; SEQ ID NO:16 and SEQ ID NO: 17), D2-15 (RIL*WW*LLL), and D2-21 (SILWW*LLF; SEQ ID NO:19), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[00077] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (VLRFLEWLLY; SEQ ID NO:21), D3-9 (VLRYFDWLL*; SEQ ID NO:23), D3-10 (VLLWFGELL*; SEQ ID NO:25), D3-16 (VL*LRLGELSLY; SEQ ID NO:27), and D3-22 (VLL***WLLL; SEQ ID NO:29), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[00078] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (*LQ*L), D4-11 (*LQ*L), D4-17 (*LR*L), and D4-23 (*LRW*L), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[00079] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (WIQLWL; SEQ ID NO:35); D5-12 (WI*WLRL; SEQ ID NO:37), D5-18 (WIQLWL; SEQ ID NO:35), and D5-24 (*RWLQL; SEQ ID NO:39), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[00080] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (V*QLV), D6-13 (V*QQLV; SEQ ID NO:41), and D6-19 (V*QWLV; SEQ ID NO:43), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[00081] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes D7-27 (*LG), and the human D gene segment further comprises a modification that replaces at least one endogenous codon of the human D gene segment in the nucleotide sequence with a histidine codon.

[00082] In one embodiment, the reading frame is a hydrophilic frame of a human D gene segment.

[00083] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (YNWND; SEQ ID NO: 45), D1-7 (YNWNY; SEQ ID NO: 47), D1-20 (YNWND; SEQ ID NO: 45), and D1-26 (YSGSYY; SEQ ID NO:49), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, 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, and a combination thereof.

[00084] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (GYCSSTSCYT; SEQ ID NO:51), D2-8 (GYCTNGVCYT; SEQ ID NO: 53), D2-15 (GYCSGGSCYS; SEQ ID NO:55), and D2-21 (AYCGGDCYS; SEQ ID NO:57), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, and a combination thereof.

[00085] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (YYDFWSGYYT; SEQ ID NO:59), D3-9 (YYDILTGYYN; SEQ ID NO:61), D3-10 (YYYGSGSYYN; SEQ ID NO:63), D3-16 (YYDYVWGSYRYT; SEQ ID NO:65), and D3-22 (YYYDSSGYYY; SEQ ID NO:67), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, and a combination thereof.

[00086] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (DYSNY; SEQ ID NO:69), D4-11 (DYSNY; SEQ ID NO:69), D4-17 (DYGDY; SEQ ID NO:71), and D4-23 (DYGGNS; SEQ ID NO:73), and the human D gene segment comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, and a combination thereof.

[00087] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (GYSYGY; SEQ ID NO:75), D5-12 (GYSGYDY; SEQ ID NO:77), D5-18 (GYSYGY; SEQ ID NO:75), and D5-24 (RDGYNY; SEQ ID NO:79), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, and a combination thereof.

[00088] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (EYSSSS; SEQ ID NO: 81), D6-13 (GYSSSWY; SEQ ID NO:83), and D6-19 (GYSSGWY; SEQ ID NO:85), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 76, and a combination thereof.

[00089] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes D7-27 (NWG), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence a histidine codon.

[00090] In one embodiment, the hydrophilic frame of the human D gene segment 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.

[00091] In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (*i.e.*, C_H1-hinge-C_H2-C_H3).

[00092] In one embodiment, a heavy chain constant region nucleotide sequence is present at an endogenous locus (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.*, at a locus different from the endogenous immunoglobulin chain locus in its genome), or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome.

[00093] In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[00094] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (*e.g.,* E or Q); 250 and 428 (*e.g.,* L or F); 252 (*e.g.,* L/Y/F/W or T), 254 (*e.g.,* S or T), and 256 (*e.g.,* S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.,* L/R/S/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.*, in an endosome where pH ranges from about 5.5 to about 6.0).

[00095] In one embodiment, the heavy chain constant region nucleotide sequence

encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[00096] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[00097] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[00098] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

[00099] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

[000100] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

[000101] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

[000102] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

[000103] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

[000104] In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1, which is incorporated by reference herein in its entirety).

[000105] In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon numbering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

[000106] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and V422I by EU).

[000107] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

[000108] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

[000109] In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[000110] In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[000111] In one embodiment, all or substantially all endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (*e.g.*, via insertion of a nucleotide sequence, *e.g.*, an exogenous nucleotide sequence, in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, *e.g.*, about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, *e.g.,* at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

[000112] In one embodiment, the genetically modified locus comprises a modification that deletes or renders non-functional all or substantially all endogenous V_H, D, and J_H gene segments; and the genomic locus comprises a genetically modified, unrearranged human heavy chain variable region nucleotide sequence comprising a substitution of at least one endogenous non-histidine codon with a histidine codon in at least one reading frame. In one embodiment, the genetically modified, unrearranged immunoglobulin heavy chain variable gene sequence is present at an endogenous location (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.,* at a locus different from the endogenous immunoglobulin chain locus in its genome), or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome.

[000113] In one embodiment, the genetically modified locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

[000114] In one embodiment, the genetically modified locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a mouse Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a mouse Adam6b gene. In one embodiment, the Adam6b gene is a human Adam6b gene.

[000115] In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized, unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

[000116] In one embodiment, the genetically modified immunoglobulin locus comprises an unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., a rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse κ locus. In one embodiment the mouse immunoglobulin light chain locus is a mouse λ locus.

[000117] In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglobulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

[000118] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (*e.g.,* at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without

the genetic modification described herein.

[000119] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (*e.g.,* pH of about 7.0 to about 7.4).

[000120] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

[000121] In one embodiment, the genetically modified immunoglobulin locus described herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, *e.g.*, antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins, which are produced by the genetically modified immunoglobulin locus described herein, when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding

protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

[000122] In one aspect, a genetically modified immunoglobulin locus of a non-human animal comprising a human V_H, D, and J_H gene segment is provided, wherein at least one human D gene segment has been inverted 5' to 3' with respect to a corresponding wild-type sequence, and wherein at least one reading frame of the inverted human D gene segment comprises one ore more histidine codon.

[000123] In one embodiment, the non-human animal is a mammal, including a rodent, *e.g.,* a mouse, a rat, or a hamster

[000124] In one embodiment, the genetically modified immunoglobulin locus is present in a germline genome.

[000125] In one embodiment, the genetically modified immunoglobulin locus encodes an immunoglobulin heavy chain variable domain comprising one or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, or 34 or more of histidine residues.

[000126] In one embodiment, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty one, at least twenty two, at least twenty three, at least twenty four, or all or substantially all of functional human D gene segments have inverted orientation with respect to corresponding wild type sequences.

[000127] In one embodiment, all or substantially all of endogenous immunoglobulin V_H, D, J_H gene segments are deleted from the immunoglobulin heavy chain locus or rendered non-functional (*e.g.,* via insertion of a nucleotide sequence, *e.g.,* exogenous nucleotide sequence, in the immunoglobulin locus or via non-functional rearrangement or inversion of all, or substantially all, endogenous immunoglobulin V_H, D, J_H segments), and the genetically modified immunoglobulin locus comprises a human V_H, D, and J_H gene segments, wherein

at least one human D gene segment is present in an inverted orientation with respect to a corresponding wild type sequence, and wherein at least one reading frame in the inverted human D gene segment comprises at least one histidine codon.

[000128] In one embodiment, the inverted human D gene segment is operably linked to a human V_H gene segment, and/or human J_H gene segment

[000129] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is selected from the group consisting of D1-1, D1-7, D1-20, D1-26, D2-2, D2-8, D2-15, D2-21, D3-3, D3-9, D3-10, D3-16, D3-22, D4-4, D4-11, D4-17, D4-23, D5-5, D5-12, D5-18, D5-24, D6-6, D6-13, D6-19, D7-27, and a combination thereof.

[000130] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D1 gene segment selected from the group consisting of D1-1, D1-7, D1-20, D1-26, and a combination thereof.

[000131] In one embodiment, the human D gene segment that is present in the inverted orientation relative a corresponding wild type sequence is a D2 gene segment selected from the group consisting of D2-2, D2-8, D2-15, D2-21, and a combination thereof.

[000132] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D3 gene segment selected from the group consisting of D3-3, D3-9, D3-10, D3-16, D3-22, and a combination thereof.

[000133] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D4 gene segment selected from the group consisting of D4-4, D4-11, D4-17, D4-23, and a combination thereof.

[000134] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D5 gene segment selected from the group consisting of D5-5, D5-12, D5-18, D5-24, and a combination thereof.

[000135] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D6 gene segment selected from the group consisting of D6-6, D6-13, D6-19, and a combination thereof.

[000136] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is D7-27.

[000137] In one embodiment, the reading frame of the human D gene segment is selected from a stop reading frame, a hydrophilic reading frame, and a hydrophobic reading frame, and at least one reading frame of the inverted human D gene segment comprises one or more histidine codon.

[000138] In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprising the inverted human D gene segment is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

[000139] In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprising the inverted human D gene segment is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

[000140] In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (*i.e.*, C_H1-hinge-C_H2-C_H3).

[000141] In one embodiment, a heavy chain constant region nucleotide sequence is present at an endogenous locus (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.*, at a locus different from the endogenous immunoglobulin chain locus in its genome), or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome.

[000142] In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000143] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (*e.g.,* E or Q); 250 and 428 (*e.g.,* L or F); 252 (*e.g.,* L/Y/F/W or T), 254 (*e.g.,* S or T), and 256 (*e.g.,* S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.,* L/R/S/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one

embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.*, in an endosome where pH ranges from about 5.5 to about 6.0).

[000144] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000145] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000146] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000147] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

[000148] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

[000149] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

[000150] In one embodiment, the heavy chain constant region nucleotide sequence

encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

[000151] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

[000152] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

[000153] In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1, incorporated by reference herein in its entirety).

[000154] In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon numbering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

[000155] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and V422I by EU).

[000156] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

[000157] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

[000158] In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[000159] In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[000160] In one embodiment, all, or substantially all, endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (*e.g.*, via insertion of a nucleotide sequence (*e.g.*, an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, *e.g.*, about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, *e.g.,* at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

[000161] In one embodiment, the genetically modified immunoglobulin heavy chain locus comprises a modification that deletes or renders non-functional, all or substantially all, endogenous V_H, D, and J_H gene segments; and the genetically modified locus comprises an unrearranged heavy chain variable region nucleotide sequence comprising at least one inverted human D gene segment as described herein wherein the unrearranged heavy chain variable region nucleotide sequence is present at an endogenous location (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.,* at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

[000162] In one embodiment, the genetically modified immunoglobulin locus comprises an

endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

[000163] In one embodiment, the genetically modified immunoglobulin locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a mouse Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a mouse Adam6b gene. In one embodiment, the Adam6b gene is a human Adam6b gene.

[000164] In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized, unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

[000165] In one embodiment, the genetically modified immunoglobulin locus comprises an unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., a rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse immunoglobulin light chain

locus is a mouse κ locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse immunoglobulin light chain locus is a mouse λ locus.

[000166] In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglobulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

[000167] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (*e.g.,* at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

[000168] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (*e.g.,* pH of about 7.0 to about 7.4).

[000169] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described

herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

[000170] In one embodiment, the genetically modified immunoglobulin locus described herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, *e.g.*, antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins as described herein when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

[000171] In one aspect, a non-human animal is provided comprising in its germline genome a genetically modified immunoglobulin locus comprising an unrearranged human heavy chain variable region nucleotide sequence, wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon.

[000172] In one embodiment, the non-human animal is a mammal, including a rodent, *e.g.,* a mouse, a rat, or a hamster.

[000173] In one embodiment, 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. In one embodiment, the 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.

[000174] In one embodiment, the human V_H gene segment is selected from the group consisting of V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23, V_H3-30, V_H3-30-3, V_H 3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31,

V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, V_H7-81, and a combination thereof.

[000175] In one embodiment, the human D gene segment is selected from the group consisting of D1-1, D1-7, D1-14, D1-20, D1-26, D2-2, D2-8, D2-15, D2-21, D3-3, D3-9, D3-10, D3-16, D3-22, D4-4, D4-11, D4-17, D4-23, D5-12, D5-5, D5-18, D5-24, D6-6, D6-13, D6-19, D6-25, D7-27, and a combination thereof.

[000176] In one embodiment, the human J_H gene segment is selected from the group consisting of J_H1, J_H2, J_H3, J_H4, J_H5, J_H6, and a combination thereof.

[000177] In one embodiment, the added or substituted histidine codon is present in the unrearranged heavy chain variable region nucleotide sequence encoding an N-terminal region, a loop 4 region, a CDR1, a CDR2, a CDR3, or a combination thereof.

[000178] In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprises 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, or 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, 40 or more, 41 or more, 42 or more, 43 or more, 44 or more, 45 or more, 46 or more, 47 or more, 48 or more, 49 or more, 50 or more, 51 or more, 52 or more, 53 or more, 54 or more, 55 or more, 56 or more, 57 or more, 58 or more, 59 or more, 60 or more, or 61 or more of histidine codons.

[000179] In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprising the inverted human D gene segment is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

[000180] In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (*i.e.*, C_H1-hinge-C_H2-C_H3).

[000181] In one embodiment, a heavy chain constant region nucleotide sequence is present at an endogenous locus (*i.e.,* where the nucleotide sequence is located in a wild-

type non-human animal) or present ectopically (*e.g.*, at a locus different from the endogenous immunoglobulin chain locus in its genome), or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome.

[000182] In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000183] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (*e.g.,* E or Q); 250 and 428 (*e.g.,* L or F); 252 (*e.g.,* L/Y/F/W or T), 254 (*e.g.,* S or T), and 256 (*e.g.,* S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.,* L/R/S/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.*, in an endosome where pH ranges from about 5.5 to about 6.0).

[000184] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000185] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000186] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence

comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000187] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

[000188] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

[000189] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

[000190] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

[000191] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

[000192] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

[000193] In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1,

which is incorporated by reference herein in its entirety).

[000194] In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon numbering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

[000195] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and V422I by EU).

[000196] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

[000197] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

[000198] In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[000199] In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[000200] In one embodiment, all or substantially all endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (*e.g.*, via insertion of a nucleotide sequence (*e.g.*, an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, *e.g.*, about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about

98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, *e.g.,* at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

[000201] In one embodiment, the genetically modified immunoglobulin heavy chain locus comprises a modification that deletes or renders, all or substantially all, non-functional endogenous V_H, D, and J_H gene segments; and the genetically modified locus comprises an unrearranged heavy chain variable region nucleotide sequence comprising one or more human V_H, D, and/or J_H gene segments having one or more histidine codons, wherein the unrearranged heavy chain variable region nucleotide sequence is present at an endogenous location (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.,* at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

[000202] In one embodiment, the genetically modified immunoglobulin locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

[000203] In one embodiment, the genetically modified immunoglobulin locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a human Adam6b gene.

[000204] In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized, unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light

chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

[000205] In one embodiment, the genetically modified immunoglobulin locus comprises an unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., a rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse κ locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse λ locus.

[000206] In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglobulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

[000207] In one embodiment, the non-human animal is heterozygous for the genetically modified immunoglobulin heavy chain locus, and the non-human animal is capable of expressing a human immunoglobulin heavy chain variable domain comprising at least one histidine residue derived predominantly from the genetically modified immunoglobulin heavy chain locus as described herein.

[000208] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (*e.g.,* at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

[000209] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH

of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (*e.g.,* pH of about 7.0 to about 7.4).

[000210] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

[000211] In one embodiment, the genetically modified immunoglobulin locus described herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, *e.g.*, antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins as described herein when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

[000212] In one aspect, a non-human animal comprising a genetically modified immunoglobulin locus is provided, wherein the genetically modified immunoglobulin locus comprises an unrearranged human heavy chain variable region nucleotide sequence, and wherein the human unrearranged heavy chain variable region nucleotide sequence comprises a substitution of at least one endogenous non-histidine codon with a histidine codon.

[000213] In one embodiment, the non-human animal is a mammal, including a rodent, *e.g.,* a mouse, a rat, or a hamster.

[000214] In one embodiment, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, 40 or more, 41 or more, 42 or more, 43 or more, 44 or more, 45 or more, 46 or more, 47 or more, 48 or more, 49 or more, 50 or more, 51 or more, 52 or more, 53 or more, 54 or more, 55 or more, 56 or more, 57 or more, 58 or more, 59 or more, 60 or more, or 61 or more of the endogenous non-histidine codons are replaced with histidine codons.

[000215] In one embodiment, the endogenous non-histone codon encodes the amino acid selected from Y, N, D, Q, S, W, and R.

[000216] In one embodiment, the substituted histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes an immunoglobulin variable domain selected from an N-terminal region, a loop 4 region, a CDR1, a CDR2, a CDR3, a combination thereof.

[000217] In one embodiment, the substituted histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes a complementary determining region (CDR) selected from a CDR1, a CDR2, a CDR3, and a combination thereof.

[000218] In one embodiment, the substituted histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes a frame region (FR) selected from FR1, FR2, FR3, FR4, and a combination thereof.

[000219] In one embodiment, the unrearranged heavy chain variable region nucleotide

sequence comprises a genetically modified human V_H gene segment, wherein one or more endogenous non-histidine codon in at least one reading frame of the human V_H gene segment has been replaced with a histidine codon.

[000220] In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a modification that replaces at least one endogenous non-histidine codon of a human V_H gene segment with a histidine codon, wherein the human V_H gene segment is selected from the group consisting of V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23, V_H3-30, V_H3-30-3, V_H 3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, V_H7-81, and a combination thereof.

[000221] In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a genetically modified human J_H gene segment, wherein one or more endogenous non-histidine codon in at least one reading frame of the human J_H gene segment has been replaced with a histidine codon.

[000222] In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a modification that replaces at least one endogenous non-histidine codon of a human J_H segment with a histidine codon, wherein the human J_H gene segment is selected from the group consisting of J_H1, J_H2, J_H3, J_H4, J_H5, J_H6, and a combination thereof.

[000223] In one embodiment, the substituted histidine codon is present in a heavy chain variable region nucleotide sequence that encodes part of a CDR3. In one embodiment, the part of CDR3 comprises an amino acid sequence derived from a reading frame of a genetically modified human D gene segment comprising a modification that replaces at least one endogenous non-histidine codon in the reading frame with a histidine codon.

[000224] In one embodiment, the endogenous non-histidine codon that is substituted with a histidine codon encodes the amino acid selected from Y, N, D, Q, S, W, and R.

[000225] In one embodiment, the substituted histidine codon is present in at least one reading frame of the human D gene segment that is most frequently observed in VELOCIMMUNE® humanized immunoglobulin mice.

[000226] In one embodiment, the reading frame of the genetically modified human D gene

segment that encodes part of CDR3 is selected from a hydrophobic frame, a stop frame, and a hydrophilic frame.

[000227] In one embodiment, the reading frame is a hydrophobic frame of a human D gene segment.

[000228] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (GTTGT; SEQ ID NO: 88), D1-7 (GITGT; SEQ ID NO: 89), D1-20 (GITGT; SEQ ID NO: 89), and D1-26 (GIVGAT; SEQ ID NO:90), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000229] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (DIVVVPAAI; SEQ ID NO:92), D2-8 (DIVLMVYAI; SEQ ID NO: 94), D2-15 (DIVVVVAAT; SEQ ID NO:95), and D2-21 (HIVVVTAI; SEQ ID NO: 97), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000230] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (ITIFGVVII; SEQ ID NO:98), D3-9 (ITIF*LVII; SEQ ID NO:99, SEQ ID NO:100), D3-10 (ITMVRGVII; SEQ ID NO:101), D3-16 (IMITFGGVIVI; SEQ ID NO:102), and D3-22 (ITMIVVVIT; SEQ ID NO:103), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon.

[000231] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (TTVT; SEQ ID NO:105), D4-11 (TTVT; SEQ ID NO:105), D4-17 (TTVT; SEQ ID NO:105), D4-23 (TTVVT; SEQ ID NO: 106) and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000232] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (VDTAMV; SEQ ID NO: 107), D5-12 (VDIVATI; SEQ ID NO:108), D5-18 (VDTAMV; SEQ ID NO:107), and D5-24 (VEMATI; SEQ ID NO:109), and the human

D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000233] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (SIAAR; SEQ ID NO:111), D6-13 (GIAAAG; SEQ ID NO:113), and D6-19 (GIAVAG; SEQ ID NO:115), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000234] In one embodiment, the hydrophobic frame comprises a nucleotide sequence that encodes human D7-27 (LTG), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000235] In one embodiment, the reading frame is a stop reading frame of a human D gene segment.

[000236] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (VQLER; SEQ ID NO:8), D1-7(V*LEL), D1-20(V*LER), D1-26 (V*WELL; SEQ ID NO:12), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000237] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (RIL**YQLLY; SEQ ID NO:14), D2-8 (RILY*WCMLY; SEQ ID NO:16 and SEQ ID NO: 17), D2-15 (RIL*WW*LLL), and D2-21 (SILWW*LLF; SEQ ID NO:19), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000238] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (VLRFLEWLLY; SEQ ID NO:21), D3-9 (VLRYFDWLL*; SEQ ID NO:23), D3-10 (VLLWFGELL*; SEQ ID NO:25), D3-16 (VL*LRLGELSLY; SEQ ID NO:27), and D3-22 (VLL***WLLL; SEQ ID NO:29), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide

sequence with a histidine codon.

[000239] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (*LQ*L), D4-11 (*LQ*L), D4-17 (*LR*L), and D4-23 (*LRW*L), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000240] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (WIQLWL; SEQ ID NO:35); D5-12 (WI*WLRL; SEQ ID NO:37), D5-18 (WIQLWL; SEQ ID NO:35), and D5-24 (*RWLQL; SEQ ID NO:39), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000241] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (V*QLV), D6-13 (V*QQLV; SEQ ID NO:41), and D6-19 (V*QWLV; SEQ ID NO:43), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000242] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes D7-27 (*LG), and the human D gene segment further comprises a modification that replaces at least one endogenous codon of the human D gene segment in the nucleotide sequence with a histidine codon.

[000243] In one embodiment, the reading frame is a hydrophilic frame of a human D gene segment.

[000244] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (YNWND; SEQ ID NO: 45), D1-7 (YNWNY; SEQ ID NO: 47), D1-20 (YNWND; SEQ ID NO: 45), and D1-26 (YSGSYY; SEQ ID NO:49), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, 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, and a combination thereof.

[000245] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (GYCSSTSCYT; SEQ ID NO:51), D2-8 (GYCTNGVCYT; SEQ ID NO: 53), D2-15 (GYCSGGSCYS; SEQ ID NO:55), and D2-21 (AYCGGDCYS; SEQ ID NO:57), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, and a combination thereof.

[000246] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (YYDFWSGYYT; SEQ ID NO:59), D3-9 (YYDILTGYYN; SEQ ID NO:61), D3-10 (YYYGSGSYYN; SEQ ID NO:63), D3-16 (YYDYVWGSYRYT; SEQ ID NO:65), and D3-22 (YYYDSSGYYY; SEQ ID NO:67), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, and a combination thereof.

[000247] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (DYSNY; SEQ ID NO:69), D4-11 (DYSNY; SEQ ID NO:69), D4-17 (DYGDY; SEQ ID NO:71), and D4-23 (DYGGNS; SEQ ID NO:73), and the human D gene segment comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, and a combination thereof.

[000248] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (GYSYGY; SEQ ID NO:75), D5-12 (GYSGYDY; SEQ ID NO:77), D5-18 (GYSYGY; SEQ ID NO:75), and D5-24 (RDGYNY; SEQ ID NO:79), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic

frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, and a combination thereof.

[000249] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (EYSSSS; SEQ ID NO: 81), D6-13 (GYSSSWY; SEQ ID NO:83), and D6-19 (GYSSGWY; SEQ ID NO:85), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 76, and a combination thereof.

[000250] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes D7-27 (NWG), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence a histidine codon.

[000251] In one embodiment, the hydrophilic frame of the human D gene segment 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.

[000252] In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprising the inverted human D gene segment is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

[000253] In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (*i.e.*, C_H1-hinge-C_H2-C_H3).

[000254] In one embodiment, a heavy chain constant region nucleotide sequence is

present at an endogenous locus (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.*, at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

[000255] In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000256] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (*e.g.,* E or Q); 250 and 428 (*e.g.,* L or F); 252 (*e.g.,* L/Y/F/W or T), 254 (*e.g.,* S or T), and 256 (*e.g.,* S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.,* L/R/S/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.*, in an endosome where pH ranges from about 5.5 to about 6.0).

[000257] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000258] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000259] In one embodiment, the heavy chain constant region nucleotide sequence

encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000260] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

[000261] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

[000262] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

[000263] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

[000264] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

[000265] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

[000266] In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the

second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1, which is incorporated by reference herein in its entirety).

[000267] In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon numbering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

[000268] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and V422I by EU).

[000269] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

[000270] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

[000271] In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[000272] In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[000273] In one embodiment, all, or substantially all, endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (*e.g.*, via insertion of a nucleotide sequence (*e.g.*, an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, *e.g.*, about 80% or more, about 85% or more,

about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, *e.g.,* at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

[000274] In one embodiment, the genetically modified locus comprises a modification that deletes or renders non-functional all or substantially all endogenous V_H, D, and J_H gene segments; and the genomic locus comprises the genetically modified, unrearranged human heavy chain variable region nucleotide sequence comprising a substitution of at least one endogenous non-histidine codon with a histidine codon in at least one reading frame. In one embodiment, the genetically modified, unrearranged immunoglobulin heavy chain variable gene sequence is present at an endogenous location (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.,* at a locus different from the endogenous immunoglobulin chain locus in its genome), or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome.

[000275] In one embodiment, the genetically modified locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

[000276] In one embodiment, the genetically modified locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a mouse Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a mouse Adam6b gene. In one embodiment, the Adam6b gene is a human Adam6b gene.

[000277] In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized,

unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

[000278] In one embodiment, the genetically modified immunoglobulin locus comprises an unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse κ locus. In one embodiment the mouse immunoglobulin light chain locus is a mouse λ locus.

[000279] In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglobulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

[000280] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (*e.g.,* at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

[000281] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain

expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (*e.g.,* pH of about 7.0 to about 7.4).

[000282] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

[000283] In one embodiment, the genetically modified immunoglobulin locus as described herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, *e.g.*, antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins as described herein, when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

[000284] In one embodiment, the non-human animal is heterozygous for the genetically modified immunoglobulin heavy chain locus, and the non-human animal is capable of expressing the human immunoglobulin heavy chain variable domain comprising at least one histidine residue derived predominantly from the genetically modified immunoglobulin heavy

chain locus as described herein.

[000285] In one aspect, a non-human animal comprising a genetically modified immunoglobulin locus comprising a human V_H, D, and J_H gene segment is provided, wherein at least one of the human D gene segment has been inverted 5' to 3' with respect to a corresponding wild-type sequence, and wherein at least one reading frame of the inverted human D gene segment comprises a histidine codon.

[000286] In one embodiment, the non-human animal is a mammal, including a rodent, *e.g.,* a mouse, a rat, or a hamster

[000287] In one embodiment, the genetically modified immunoglobulin locus is present in a germline genome.

[000288] In one embodiment, wherein the reading frame of the inverted human D gene segment comprises one or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, or 34 or more of histidine codons.

[000289] In one embodiment, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty one, at least twenty two, at least twenty three, at least twenty four, or all or substantially all of functional human D gene segments have inverted orientation with respect to corresponding wild type sequences.

[000290] In one embodiment, all or substantially all of endogenous immunoglobulin V_H, D, J_H gene segments are deleted from the immunoglobulin heavy chain locus or rendered non-functional (*e.g.,* via insertion of a nucleotide sequence, *e.g.,* exogenous nucleotide sequence, in the immunoglobulin locus or via non-functional rearrangement or inversion of all, or substantially all, endogenous immunoglobulin V_H, D, J_H segments), and the genetically modified immunoglobulin locus comprises a human V_H, D, and J_H gene segments, wherein at least one of the human D gene segment is present in an inverted orientation with respect to corresponding wild type sequences, and wherein at least one reading frame of the inverted human D gene segment comprises at least one histidine codon.

[000291] In one embodiment, the inverted human D gene segment is operably linked to a

human V_H gene segment, and/or human J_H gene segment

[000292] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is selected from the group consisting of D1-1, D1-7, D1-20, D1-26, D2-2, D2-8, D2-15, D2-21, D3-3, D3-9, D3-10, D3-16, D3-22, D4-4, D4-11, D4-17, D4-23, D5-5, D5-12, D5-18, D5-24, D6-6, D6-13, D6-19, D7-27, and a combination thereof.

[000293] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D1 gene segment selected from the group consisting of D1-1, D1-7, D1-20, D1-26, and a combination thereof.

[000294] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequences is a D2 gene segment selected from the group consisting of D2-2, D2-8, D2-15, D2-21, and a combination thereof.

[000295] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D3 gene segment selected from the group consisting of D3-3, D3-9, D3-10, D3-16, D3-22, and a combination thereof.

[000296] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D4 gene segment selected from the group consisting of D4-4, D4-11, D4-17, D4-23, and a combination thereof.

[000297] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D5 gene segment selected from the group consisting of D5-5, D5-12, D5-18, D5-24, and a combination thereof.

[000298] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D6 gene segment selected from the group consisting of D6-6, D6-13, D6-19, and a combination thereof.

[000299] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is D7-27.

[000300] In one embodiment, the reading frame of the human D gene segment is selected from a stop reading frame, a hydrophilic reading frame, a hydrophobic reading frame, and a combination thereof, wherein at least one reading frame of the inverted human D gene segment comprises a histidine codon.

[000301] In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprising the inverted human D gene segment is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

[000302] In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (*i.e.*, C_H1-hinge-C_H2-C_H3).

[000303] In one embodiment, a heavy chain constant region nucleotide sequence is present at an endogenous locus (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.*, at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

[000304] In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000305] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (*e.g.,* E or Q); 250 and 428 (*e.g.,* L or F); 252 (*e.g.,* L/Y/F/W or T), 254 (*e.g.,* S or T), and 256 (*e.g.,* S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.,* L/R/S/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.*, in an endosome where pH ranges from about 5.5 to about 6.0).

[000306] In one embodiment, the heavy chain constant region nucleotide sequence

encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000307] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000308] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000309] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

[000310] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

[000311] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

[000312] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

[000313] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

[000314] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

[000315] In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1, incorporated by reference herein in its entirety).

[000316] In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon numbering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

[000317] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and V422I by EU).

[000318] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

[000319] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

[000320] In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[000321] In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[000322] In one embodiment, all or substantially all endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (*e.g.*, via insertion of a nucleotide sequence (*e.g.*, an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, *e.g.*, about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, *e.g.,* at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

[000323] In one embodiment, the genetically modified immunoglobulin heavy chain locus comprises a modification that deletes or renders, all or substantially all, non-functional endogenous V_H, D, and J_H gene segments; and the genetically modified locus comprises an unrearranged heavy chain variable region nucleotide sequence comprising at least one inverted human D gene segment as described herein wherein the unrearranged heavy chain variable region nucleotide sequence is present at an endogenous location (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.,* at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

[000324] In one embodiment, the genetically modified immunoglobulin locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

[000325] In one embodiment, the genetically modified immunoglobulin locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a mouse Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a mouse Adam6b gene. In one embodiment, the Adam6b gene is a human

Adam6b gene.

[000326] In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized, unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

[000327] In one embodiment, the genetically modified immunoglobulin locus comprises an unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., a rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse κ locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse immunoglobulin light chain locus is a mouse λ locus.

[000328] In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglobulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

[000329] In one embodiment, the non-human animal is heterozygous for the genetically modified immunoglobulin heavy chain locus, and the non-human animal is capable of

expressing the human immunoglobulin heavy chain variable domain comprising at least one histidine residue derived predominantly from the genetically modified immunoglobulin heavy chain locus as described herein.

[000330] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (*e.g.,* at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

[000331] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (*e.g.,* pH of about 7.0 to about 7.4).

[000332] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

[000333] In one embodiment, the genetically modified immunoglobulin locus described

herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, *e.g.*, antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins as described herein when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

[000334] In one aspect, a non-human animal that is capable of expressing an antigen-binding protein with enhanced pH-dependent recyclability and/or enhanced serum half-life are provided, wherein the non-human animal comprises in its germline genome an unrearranged human immunoglobulin heavy chain variable region nucleotide sequence, wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon as described herein.

[000335] In one embodiment, the antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (*e.g.,* at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

[000336] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a

heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (*e.g.,* pH of about 7.0 to about 7.4).

[000337] In one embodiment, the antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

[000338] In one embodiment, the genetically modified immunoglobulin locus described herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, *e.g.*, antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins as described herein when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

[000339] In one aspect, a targeting construct is provided, comprising 5' and 3' targeting arms homologous to a genomic D region or genomic V and J region of a non-human animal, wherein at least one V_H, D, or J_H gene segment comprises any of the modifications as described herein, *e.g.*, an addition of at least one histidine codon, a substitution of at least one endogenous non-histidine codon into a histidine codon, and/or inversion of at least one functional D gene segment with respect to a corresponding wild type sequence.

[000340] In one aspect, a hybridoma or quadroma is provided that is derived from a cell of

any of the non-human animal as described herein. In one embodiment, the non-human animal is a rodent, *e.g.*, a mouse, a rat, or a hamster.

[000341] In one aspect, pluripotent, induced pluripotent, or totipotent stem cells derived form a non-human animal comprising the various genomic modifications of the described invention are provided. In a specific embodiment, the pluripotent, induced pluripotent, or totipotent stem cells are mouse or rat embryonic stem (ES) cells. In one embodiment, the pluripotent, induced pluripotent, or totipotent stem cells have an XX karyotype or an XY karyotype. In one embodiment, the pluripotent or induced pluripotent stem cells are hematopoietic stem cells.

[000342] In one aspect, cells that comprise a nucleus containing a genetic modification as described herein are also provided, *e.g.,* a modification introduced into a cell by pronuclear injection. In one embodiment, the pluripotent, induced pluripotent, or totipotent stem cells comprise a genetically modified immunoglobulin genomic locus, wherein the genomic locus comprises, from 5' to 3', (1) an FRT recombination site, (2) human V_H gene segments, (3) a mouse adam6 gene, (4) a loxP recombination site, (5) histidine-substituted human D gene segments, (6) human J_H gene segments, followed by (7) a mouse E_i (intronic enhancer), and (8) a mouse IgM constant region nucleotide sequence.

[000343] In one aspect, a lymphocyte isolated from a genetically modified non-human animal as described herein is provided. In one embodiment, the lymphocyte is a B cell, wherein the B cell comprises an immunoglobulin genomic locus comprising an unrearranged heavy chain variable region nucleotide sequence wherein the unrearranged heavy chain variable gene sequence comprises an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon.

[000344] In one aspect, a lymphocyte isolated from a genetically modified non-human animal as described herein is provided. In one embodiment, the lymphocyte is a B cell, wherein the B cell comprises an immunoglobulin locus that comprises a human V, D, and J gene segment, wherein at least one of the human D gene segment has been inverted 5' to 3' with respect to wild-type sequences, and wherein at least one reading frame of the inverted human D gene segment encodes at least one histidine residue. In one embodiment, the B cell is capable of producing an antigen-binding protein comprising the genetically modified heavy chain variable domain as described herein. In one embodiment, the genetically modified heavy chain variable domain as described herein is operably linked to a heavy chain constant region amino acid sequence.

[000345] In one aspect, a B cell population is provided that are capable of expressing an antigen-binding protein comprising at least one histidine residue in a heavy chain variable domain, wherein the B cell population comprises any genetic modifications as described herein. In one embodiment, the at least one histidine residue is present in a heavy chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the at least one histidine residue is present in CDR3.

[000346] In one aspect, a B cell population is provided that are capable of expressing an antigen-binding protein with enhanced serum half-life and/or enhanced pH-dependent recyclability, wherein the B cell population comprises any genetic modifications as described herein.

[000347] In one aspect, a method for making a non-human animal comprising a genetically modified immunoglobulin heavy chain variable locus is provided, comprising:

[000348] (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 (*e.g.*, via insertion of a nucleotide sequence, *e.g.*, an exogenous nucleotide sequence, in the immunoglobulin locus or via non-functional rearrangement or inversion of endogenous V_H, D, J_H segments); and

[000349] (b) placing in the genome an unrearranged heavy chain variable region nucleotide sequence, wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon as described herein.

[000350] In one embodiment, the non-human animal is a mammal, including a rodent, e.g., a mouse, a rat, or a hamster.

[000351] In one embodiment, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, 40 or more, 41 or more, 42 or more, 43 or more, 44 or more, 45 or more, 46 or more, 47 or more, 48 or more, 49 or more, 50 or more, 51 or more, 52 or more, 53 or more, 54 or more, 55 or more, 56 or more, 57 or more, 58 or more, 59 or more, 60 or more, or 61 or more of the endogenous non-histidine codons are replaced with

histidine codons.

[000352] In one embodiment, the endogenous non-histone codon encodes the amino acid selected from Y, N, D, Q, S, W, and R.

[000353] In one embodiment, the added or substituted histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes an immunoglobulin variable domain selected from an N-terminal region, a loop 4 region, a CDR1, a CDR2, a CDR3, a combination thereof.

[000354] In one embodiment, the added substituted histidine codon histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes a complementary determining region (CDR) selected from a CDR1, a CDR2, a CDR3, and a combination thereof.

[000355] In one embodiment, the added or substituted histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes a frame region (FR) selected from FR1, FR2, FR3, FR4, and a combination thereof.

[000356] In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprises a genetically modified human V_H gene segment, wherein one or more endogenous non-histidine codon in at least one reading frame of the human V_H gene segment has been replaced with a histidine codon.

[000357] In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a modification that replaces at least one endogenous non-histidine codon of a human V_H gene segment with a histidine codon, wherein the human V_H gene segment is selected from the group consisting of V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23, V_H3-30, V_H3-30-3, V_H 3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, V_H7-81, and a combination thereof.

[000358] In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a genetically modified human J_H gene segment, wherein one or more endogenous non-histidine codon in at least one reading frame of the human J_H gene segment has been replaced with a histidine codon.

[000359] In one embodiment, the human unrearranged heavy chain variable region

nucleotide sequence comprises a modification that replaces at least one endogenous non-histidine codon of a human J_H segment with a histidine codon, wherein the human J_H gene segment is selected from the group consisting of J_H1, J_H2, J_H3, J_H4, J_H5, J_H6, and a combination thereof.

[000360] In one embodiment, the added or substituted histidine codon is present in a heavy chain variable region nucleotide sequence that encodes part of a CDR3. In one embodiment, the part of CDR3 comprises an amino acid sequence derived from a reading frame of a genetically modified human D gene segment comprising a modification that replaces at least one endogenous non-histidine codon in the reading frame with a histidine codon.

[000361] In one embodiment, the endogenous non-histidine codon that is substituted with a histidine codon encodes the amino acid selected from Y, N, D, Q, S, W, and R.

[000362] In one embodiment, the added or substituted histidine codon is present in at least one reading frame of the human D gene segment that is most frequently observed in VELOCIMMUNE® humanized immunoglobulin mice.

[000363] In one embodiment, the reading frame of the genetically modified human D gene segment that encodes part of CDR3 is selected from a hydrophobic frame, a stop frame, and a hydrophilic frame.

[000364] In one embodiment, the reading frame is a hydrophobic frame of a human D gene segment.

[000365] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (GTTGT; SEQ ID NO: 88), D1-7 (GITGT; SEQ ID NO: 89), D1-20 (GITGT; SEQ ID NO: 89), and D1-26 (GIVGAT; SEQ ID NO:90), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000366] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (DIVVVPAAI; SEQ ID NO:92), D2-8 (DIVLMVYAI; SEQ ID NO: 94), D2-15 (DIVVVVAAT; SEQ ID NO:95), and D2-21 (HIVVVTAI; SEQ ID NO: 97), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000367] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (ITIFGVVII; SEQ ID NO:98), D3-9 (ITIF*LVII; SEQ ID NO:99, SEQ ID NO:100), D3-10 (ITMVRGVII; SEQ ID NO:101), D3-16 (IMITFGGVIVI; SEQ ID NO:102), and D3-22 (ITMIVVVIT; SEQ ID NO:103), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon.

[000368] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (TTVT; SEQ ID NO:105), D4-11 (TTVT; SEQ ID NO:105), D4-17 (TTVT; SEQ ID NO:105), D4-23 (TTVVT; SEQ ID NO: 106) and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000369] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (VDTAMV; SEQ ID NO: 107), D5-12 (VDIVATI; SEQ ID NO:108), D5-18 (VDTAMV; SEQ ID NO:107), and D5-24 (VEMATI; SEQ ID NO:109), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000370] In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (SIAAR; SEQ ID NO:111), D6-13 (GIAAAG; SEQ ID NO:113), and D6-19 (GIAVAG; SEQ ID NO:115), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000371] In one embodiment, the hydrophobic frame comprises a nucleotide sequence that encodes human D7-27 (LTG), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000372] In one embodiment, the reading frame is a stop reading frame of a human D gene segment.

[000373] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the

group consisting of D1-1 (VQLER; SEQ ID NO:8), D1-7(V*LEL), D1-20(V*LER), D1-26 (V*WELL; SEQ ID NO:12), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000374] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (RIL**YQLLY; SEQ ID NO:14), D2-8 (RILY*WCMLY; SEQ ID NO:16 and SEQ ID NO: 17), D2-15 (RIL*WW*LLL), and D2-21 (SILWW*LLF; SEQ ID NO:19), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000375] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (VLRFLEWLLY; SEQ ID NO:21), D3-9 (VLRYFDWLL*; SEQ ID NO:23), D3-10 (VLLWFGELL*; SEQ ID NO:25), D3-16 (VL*LRLGELSLY; SEQ ID NO:27), and D3-22 (VLL***WLLL; SEQ ID NO:29), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000376] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (*LQ*L), D4-11 (*LQ*L), D4-17 (*LR*L), and D4-23 (*LRW*L), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000377] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (WIQLWL; SEQ ID NO:35); D5-12 (WI*WLRL; SEQ ID NO:37), D5-18 (WIQLWL; SEQ ID NO:35), and D5-24 (*RWLQL; SEQ ID NO:39), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000378] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (V*QLV), D6-13 (V*QQLV; SEQ ID NO:41), and D6-19 (V*QWLV; SEQ ID NO:43), and the human D gene segment further comprises a modification that

replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

[000379] In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes D7-27 (*LG), and the human D gene segment further comprises a modification that replaces at least one endogenous codon of the human D gene segment in the nucleotide sequence with a histidine codon.

[000380] In one embodiment, the reading frame is a hydrophilic frame of a human D gene segment.

[000381] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (YNWND; SEQ ID NO: 45), D1-7 (YNWNY; SEQ ID NO: 47), D1-20 (YNWND; SEQ ID NO: 45), and D1-26 (YSGSYY; SEQ ID NO:49), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, 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, and a combination thereof.

[000382] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (GYCSSTSCYT; SEQ ID NO:51), D2-8 (GYCTNGVCYT; SEQ ID NO: 53), D2-15 (GYCSGGSCYS; SEQ ID NO:55), and D2-21 (AYCGGDCYS; SEQ ID NO:57), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, and a combination thereof.

[000383] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (YYDFWSGYYT; SEQ ID NO:59), D3-9 (YYDILTGYYN; SEQ ID NO:61), D3-10 (YYYGSGSYYN; SEQ ID NO:63), D3-16 (YYDYVWGSYRYT; SEQ ID NO:65), and D3-22 (YYYDSSGYYY; SEQ ID NO:67), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame

comprises a nucleotide sequence encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, and a combination thereof.

[000384] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (DYSNY; SEQ ID NO:69), D4-11 (DYSNY; SEQ ID NO:69), D4-17 (DYGDY; SEQ ID NO:71), and D4-23 (DYGGNS; SEQ ID NO:73), and the human D gene segment comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, and a combination thereof.

[000385] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (GYSYGY; SEQ ID NO:75), D5-12 (GYSGYDY; SEQ ID NO:77), D5-18 (GYSYGY; SEQ ID NO:75), and D5-24 (RDGYNY; SEQ ID NO:79), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, and a combination thereof.

[000386] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (EYSSSS; SEQ ID NO: 81), D6-13 (GYSSSWY; SEQ ID NO:83), and D6-19 (GYSSGWY; SEQ ID NO:85), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 76, and a combination thereof.

[000387] In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes D7-27 (NWG), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence a histidine codon.

[000388] In one embodiment, the hydrophilic frame of the human D gene segment 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.

[000389] In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprising the inverted human D gene segment is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

[000390] In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (*i.e.*, C_H1-hinge-C_H2-C_H3).

[000391] In one embodiment, a heavy chain constant region nucleotide sequence is present at an endogenous locus (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.*, at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

[000392] In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000393] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (*e.g.,* E or Q); 250 and 428 (*e.g.,* L or F); 252 (*e.g.,* L/Y/F/W or T), 254 (*e.g.,* S or T), and 256 (*e.g.,* S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.,* L/R/S/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S)

modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.*, in an endosome where pH ranges from about 5.5 to about 6.0).

[000394] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000395] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000396] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000397] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

[000398] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

[000399] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

[000400] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation

selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

[000401] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

[000402] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

[000403] In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1, which is incorporated by reference herein in its entirety).

[000404] In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon numbering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

[000405] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and V422I by EU).

[000406] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

[000407] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

[000408] In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[000409] In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[000410] In one embodiment, all or substantially all endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (*e.g.*, via insertion of a nucleotide sequence (*e.g.*, an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, *e.g.*, about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, *e.g.,* at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

[000411] In one embodiment, the genetically modified locus comprises a modification that deletes or renders non-functional all or substantially all endogenous V_H, D, and J_H gene segments; and the genomic locus comprises the genetically modified, unrearranged human heavy chain variable region nucleotide sequence comprising a substitution of at least one endogenous non-histidine codon with a histidine codon in at least one reading frame. In one embodiment, the genetically modified, unrearranged immunoglobulin heavy chain variable gene sequence is present at an endogenous location (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.,* at a locus different from the endogenous immunoglobulin chain locus in its genome), or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome.

[000412] In one embodiment, the genetically modified locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

[000413] In one embodiment, the genetically modified locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a mouse Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a mouse Adam6b gene. In one embodiment, the Adam6b gene is a human Adam6b gene.

[000414] In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized, unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

[000415] In one embodiment, the genetically modified immunoglobulin locus comprises an unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., a rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse κ locus. In one embodiment the mouse immunoglobulin light chain locus is

a mouse λ locus.

[000416] In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglobulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

[000417] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (*e.g.,* at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

[000418] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (*e.g.,* pH of about 7.0 to about 7.4).

[000419] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or

both as compared with a wild-type antigen-binding protein without the genetic modification.

[000420] In one embodiment, the genetically modified immunoglobulin locus described herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, *e.g.*, antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins as described herein when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

[000421] In one aspect, a method for making a non-human animal comprising a genetically modified immunoglobulin heavy chain variable locus is provided, comprising:

[000422] (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 (*e.g.*, via insertion of a nucleotide sequence (*e.g.*, an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement or inversion of endogenous V_H, D, J_H segments); and

[000423] (b) placing in the genome a human V_H, D, and J_H gene segment, wherein at least one of the human D gene segment has been inverted 5' to 3' with respect to a corresponding wild-type sequence, and wherein at least one reading frame of the inverted human D gene segment comprises a histidine codon.

[000424] In one embodiment, the non-human animal is a mammal, including a rodent, *e.g.,* a mouse, a rat, or a hamster

[000425] In one embodiment, the genetically modified immunoglobulin locus is present in a germline genome.

[000426] In one embodiment, the genetically modified immunoglobulin locus encodes an immunoglobulin heavy chain variable domain comprising one or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12

or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, or 34 or more of histidine residues.

[000427] In one embodiment, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty one, at least twenty two, at least twenty three, at least twenty four, or all or substantially all of functional human D gene segments have inverted orientation with respect to corresponding wild type sequences.

[000428] In one embodiment, all or substantially all of endogenous immunoglobulin V_H, D, J_H gene segments are deleted from the immunoglobulin heavy chain locus or rendered non-functional (*e.g.,* via insertion of a nucleotide sequence, *e.g.,* exogenous nucleotide sequence, in the immunoglobulin locus or via non-functional rearrangement or inversion of all, or substantially all, endogenous immunoglobulin V_H, D, J_H segments), and the genetically modified immunoglobulin locus comprises a human V_H, D, and J_H gene segments, wherein at least one of the human D gene segment is present in an inverted orientation with respect to a corresponding wild type sequence, and wherein at least one reading frame in the inverted human D gene segment comprises at least one histidine codon.

[000429] In one embodiment, the inverted human D gene segment is operably linked to a human V_H gene segment, and/or human J_H gene segment

[000430] In one embodiment, the human D gene segment that is present in the inverted orientation relative to wild type sequences is selected from the group consisting of D1-1, D1-7, D1-20, D1-26, D2-2, D2-8, D2-15, D2-21, D3-3, D3-9, D3-10, D3-16, D3-22, D4-4, D4-11, D4-17, D4-23, D5-5, D5-12, D5-18, D5-24, D6-6, D6-13, D6-19, D7-27, and a combination thereof.

[000431] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D1 gene segment selected from the group consisting of D1-1, D1-7, D1-20, D1-26, and a combination thereof.

[000432] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D2 gene segment selected from the group consisting of D2-2, D2-8, D2-15, D2-21, and a combination thereof.

[000433] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D3 gene segment selected from the group consisting of D3-3, D3-9, D3-10, D3-16, D3-22, and a combination thereof.

[000434] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D4 gene segment selected from the group consisting of D4-4, D4-11, D4-17, D4-23, and a combination thereof.

[000435] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D5 gene segment selected from the group consisting of D5-5, D5-12, D5-18, D5-24, and a combination thereof.

[000436] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D6 gene segment selected from the group consisting of D6-6, D6-13, D6-19, and a combination thereof.

[000437] In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is D7-27.

[000438] In one embodiment, the reading frame of the human D gene segment is selected from a stop reading frame, a hydrophilic reading frame, a hydrophobic reading frame, and a combination thereof.

[000439] In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprising the inverted human D gene segment is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

[000440] In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (*i.e.*, C_H1-hinge-C_H2-C_H3).

[000441] In one embodiment, a heavy chain constant region nucleotide sequence is present at an endogenous locus (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.*, at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

[000442] In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000443] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (*e.g.,* E or Q); 250 and 428 (*e.g.,* L or F); 252 (*e.g.,* L/Y/F/W or T), 254 (*e.g.,* S or T), and 256 (*e.g.,* S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.,* L/R/S/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.*, in an endosome where pH ranges from about 5.5 to about 6.0).

[000444] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000445] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000446] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000447] In one embodiment, the heavy chain constant region nucleotide sequence

encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

[000448] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

[000449] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

[000450] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

[000451] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

[000452] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

[000453] In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1, which is incorporated by reference herein in its entirety).

[000454] In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon

numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon numbering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

[000455] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and V422I by EU).

[000456] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

[000457] In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

[000458] In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[000459] In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[000460] In one embodiment, all or substantially all endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (*e.g.*, via insertion of a nucleotide sequence (*e.g.*, an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, *e.g.*, about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, *e.g.,* at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

[000461] In one embodiment, the genetically modified immunoglobulin heavy chain locus comprises a modification that deletes or renders, all or substantially all, non-functional endogenous V_H, D, and J_H gene segments; and the genetically modified locus comprises an unrearranged heavy chain variable region nucleotide sequence comprising at least one inverted human D gene segment as described herein wherein the unrearranged heavy chain variable region nucleotide sequence is present at an endogenous location (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.,* at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

[000462] In one embodiment, the genetically modified immunoglobulin locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

[000463] In one embodiment, the genetically modified immunoglobulin locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a mouse Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a mouse Adam6b gene. In one embodiment, the Adam6b gene is a human Adam6b gene.

[000464] In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized, unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

[000465] In one embodiment, the genetically modified immunoglobulin locus comprises an

unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., a rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse κ locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse immunoglobulin light chain locus is a mouse λ locus.

[000466] In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglobulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

[000467] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (*e.g.,* at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

[000468] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an

acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (*e.g.,* pH of about 7.0 to about 7.4).

[000469] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

[000470] In one embodiment, the genetically modified immunoglobulin locus described herein comprises an enriched B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, *e.g.*, antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins as described herein, when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

[000471] In one aspect, a method for making a non-human animal that is capable of producing an immunoglobulin heavy chain variable domain with enhanced serum half-life and/or enhanced pH-dependent recyclability is provided, comprising

[000472] (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 (*e.g.*, via insertion of a nucleotide sequence (*e.g.*, an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement or inversion of endogenous V_H, D, J_H segments); and

[000473] (b) placing in the genome an unrearranged human heavy chain variable region nucleotide sequence, wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon, and wherein an antigen-binding protein comprising the immunoglobulin heavy chain variable domain produced by the non-human animal exhibits enhanced serum half-life and/or enhanced pH-dependent recyclability as compared to a wild-type immunoglobulin heavy chain domain.

[000474] In one embodiment, the non-human animal, upon contact with an antigen, can produce an enriched population of B cell repertoire that expresses an antigen-binding protein with enhanced serum half-life and/or enhanced pH-dependent recyclability, wherein the enriched B cell population comprises any genetic modifications as described herein.

[000475] In one embodiment, an antigen-binding protein produced by the genetically modified non-human animal is characterized by sufficient affinity to an antigen of interest at a neutral pH (*e.g.*, pH of about 7.0 to about 7.4) and enhanced dissociation of the antibody from an antigen-antigen-binding protein complex at a pH less than the neutral pH (*e.g.,* at an endosomal pH, *e.g.* pH of about 5.5 to 6.0).

[000476] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) as compared to

the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (*e.g.,* pH of about 7.0 to about 7.4).

[000477] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

[000478] In one embodiment, the genetically modified immunoglobulin locus described herein comprises a an enriched B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, *e.g.*, antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins as described herein when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

[000479] In one embodiment, the antigen-binding protein comprises an immunoglobulin heavy chain variable domain that is capable of specifically binding an antigen of interest with an affinity (K_D) lower than 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, and 10^{-12} at a neutral pH (*e.g.*, pH of about 7.0 to about 7.4).

[000480] In one aspect, a method for obtaining an antigen-binding protein with enhanced recyclability and/or improved serum half-life is provided, comprising:

[000481] (a) immunizing a non-human animal having a genetically modified immunoglobulin locus as described herein wherein the non-human animal comprises an unrearranged human heavy chain variable region nucleotide sequence comprising an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon;

[000482] (b) allowing the non-human animal to mount an immune response;

[000483] (c) harvesting a lymphocyte (*e.g.*, a B cell) from the immunized non-human animal;

[000484] (d) fusing the lymphocyte with a myeloma cell to form a hybridoma cell, and

[000485] (e) obtaining an antigen-binding protein produced by the hybridoma cell, wherein the antigen-binding protein exhibits enhanced recyclability and/or serum stability.

[000486] In one aspect, a genetically modified immunoglobulin heavy chain locus obtainable by any of the methods as described herein is provided.

[000487] In one aspect, a genetically modified non-human animal obtainable by any of the methods as described herein is provided.

[000488] In various embodiments, the non-human animal is a mammal. In one embodiment, the mammal is a rodent, *e.g.*, a mouse, a rat, or a hamster.

[000489] In various embodiments, the genetically modified immunoglobulin loci as described herein are present in the germline genome of a non-human animal, *e.g.*, a mammal, e.g., a rodent, *e.g.*, a mouse, a rat, or a hamster.

BRIEF DESCRIPTION OF THE DRAWINGS

[000490] **FIGs. 1A and 1B** illustrate the amino acid sequences encoded by the three reading frames (*i.e.*, stop, hydrophilic, and hydrophobic reading frames) of human D gene segments (D) and the amino acid sequences encoded by the three reading frames of histidine-substituted human D gene segments (HD). Introduction of histidine codons (typed in bold) in the hydrophilic reading frame also changed many stop codons in the stop reading frame to Ser codons (typed in bold) but introduced few changes in the hydrophobic reading frame. The "*" symbol represents a stop codon, and the comma between the two SEQ ID NOs indicates that there are two amino acid sequences separated by the stop codon.

[000491] **FIG. 2** illustrates schemes for targeting pLMa0174 containing a spectinomycin selection cassette into the 5' end of MAID 1116 (Step 1. BHR (Spec)). In Step 1, a chloramphenicol selection cassette, a neomycin selection cassette, a loxP site, two V_H gene segments (hV_H1-3 and hV_H1-2), the human Adam6 gene, all of which are located upstream of hV_H6-1, were deleted from the clone and replaced by a spectinomycin cassette to yield the VI433 clone. In Step 2 (BHR (Hyg + Spec)), pNTu0002 containing a hygromycin cassette flanked by FRT sites was targeted into a region comprising human immunoglobulin D gene segments. Via Step 2, all human D gene segments were deleted from VI433 and replaced

with the hygromycin cassette to yield MAID6011 VI 434 (clone 1).

[000492] **FIG. 3** illustrates schemes for assembling histidine-substituted human D gene segments via sequential ligation.

[000493] **FIG. 4** illustrates the introduction of pre-assembled, histidine-substituted human D gene segments containing a neomycin cassette into a region between the most D-proximal V_H gene segment (V_H 6-1) and the most D-proximal J_H gene segment (J_H1) via enzyme-mediated digestion (PI-SceI and I-CeuI) and ligation. This process removes the hygromycin cassette from MAID 6011 VI434 and introduces pre-assembled human histidine-substituted D gene segments into the clone. Bacterial cells comprising a successfully targeted clone are selected based on both neomycin and spectinomycin resistance. The resulting clone (MAID6012 VI469) comprises, from 5' to 3', (1) a spectinomycin selection cassette, (2) a 50 kb arm comprising a human V_H gene segment (V_H 6-1), (3) a neomycin cassette flanked by loxP sites, (4) human D gene segments containing histidine substitutions (HD 1.1-6.6 (9586bp; SEQ ID NO: 1), HD 1.7-6.13 (9268bp; SEQ ID NO: 2), HD 1.14-6.19 (9441bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592bp; SEQ ID NO: 4)), (5) about 25 kb of a genomic region containing human J_H gene segments, (6) a mouse E_i sequence (SEQ ID NO: 5; an intronic enhancer that promotes V_H to DJ_H rearrangement in developing B cells), and (7) a mouse IgM constant region nucleotide sequence (mIgM exon 1; SEQ ID NO: 7).

[000494] **FIG. 5** illustrates schemes for deleting the human immunoglobulin heavy chain D gene region from the MAID 1460 heterozygous ES cells by targeting the 129 strain-derived chromosome of MAID 1460 het with the hygromycin selection cassette in MAID 6011 VI434.

[000495] **FIG 6** shows a list of primers and probes used to confirm a loss of allele (LOA), a gain of allele (GOA), or a parental allele (Parental) in the screening assays for identifying MAID 6011.

[000496] **FIG. 7** illustrates schemes for constructing MAID 6012 het by targeting MAID 6011 heterozygous ES cells with MAID 6012 VI469. Electroporation of the MAID 6012 VI469 construct into the MAID 6011 heterozygous ES cells yielded MAID 6012 heterozygous ES cells in which the 129 strain-derived chromosome is modified to contain, from 5' to 3' direction, an FRT site, human V_H gene segments, a mouse genomic region comprising adam6 genes, a floxed neomycin selection cassette, human D gene segments comprising histidine substitutions (HD 1.1-6.6 (9586bp; SEQ ID NO: 1), HD 1.7-6.13 (9268bp; SEQ ID NO: 2), HD 1.14-6.19 (9441bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592bp; SEQ ID

NO: 4)), human J_H gene segments, a mouse E_i sequence (SEQ ID NO: 5; an intronic enhancer that promotes V_H to DJ_H rearrangement in developing B cells), and a mouse IgM constant region nucleotide sequence (mIgM exon 1; SEQ ID NO: 7).

[000497] **FIG. 8** shows a list of primers and probes used to confirm a loss of allele (LOA), a gain of allele (GOA), or a parental allele (Parental) in the screening assay for identifying MAID 6012.

[000498] **FIG. 9** illustrates schemes for removing a neomycin cassette from MAID 6012 heterozygous ES cells. Electroporation of a Cre-expressing plasmid into the MAID 6012 ES cells lead to recombination and deletion of the floxed neomycin cassette, yielding MAID 6013 heterozygous ES cells.

[000499] **FIGs. 10A-10E** illustrate human D gene segment nucleotide sequences with translations for each of the six reading frames, *i.e.*, three reading frames for direct 5' to 3' orientation and three reading frames for inverted orientation (3' to 5' orientation). The "*" symbol represents a stop codon, and the comma between two SEQ ID NOs indicates that there are two amino acid sequences separated by the stop codon.

[000500] **FIGs. 11-13** illustrate mRNA sequences and their encoded protein sequences expressed by 6013 F0 heterozygous mice, which comprise histidine-substituted human D gene segments (HD 1.1-6.6 (9586bp; SEQ ID NO: 1), HD 1.7-6.13 (9268bp; SEQ ID NO: 2), HD 1.14-6.19 (9441bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592bp; SEQ ID NO: 4)) in the immunoglobulin heavy chain locus in their 129 strain-derived chromosome. The boxed sequences in each figure indicate the presence of histidine codons in the CDR3 sequences derived from the genetically modified immunoglobulin heavy chain locus comprising the histidine-substituted human D gene segments. FWR represents frame region and CDR represents complementarity determining region. In the alignment, the dot "." indicates a sequence identical to the query sequence, and the dash "-" indicates a gap in the sequence.

[000501] **FIG. 14** illustrates histidine incorporation frequency in immunoglobulin heavy chain CDR3 sequences. The X-axis represents the number of histidine codons appeared in each CDR3 sequence, and the Y-axis represents the corresponding proportion of reads. The "6013 F0 het" indicates CDR3 sequences expressed by the 6013 heterozygous mice comprising histidine-substituted D gene segments. The "VI3-Adam6" indicates CDR3 sequences obtained from control mice comprising human V_H, D, and J_H gene segments without the histidine modification as described herein. The "ASAP" indicates CDR3 sequences obtained from the Regeneron antibody database, which was used as another

control.

DETAILED DESCRIPTION OF THE INVENTION

[000502] This invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention is defined by the claims.

[000503] Unless defined otherwise, all terms and phrases used herein include the meanings that the terms and phrases have attained in the art, unless the contrary is clearly indicated or clearly apparent from the context in which the term or phrase is used. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, particular methods and materials are now described. All publications mentioned are hereby incorporated by reference

Definitions

[000504] The term "complementary determining region" or "CDR," as used herein, includes an amino acid sequence encoded by a nucleic acid sequence of an organism's immunoglobulin genes that normally (*i.e.,* in a wild type animal) appears between two framework regions in a variable region of a light or a heavy chain of an immunoglobulin molecule (*e.g.,* an antibody or a T cell receptor). A CDR can be encoded by, for example, a germline sequence or a rearranged sequence, and, for example, by a naïve or a mature B cell or a T cell. A CDR can be somatically mutated (*e.g.,* vary from a sequence encoded in an animal's germline), humanized, and/or modified with amino acid substitutions, additions, or deletions. In some circumstances (*e.g.,* for a CDR3), CDRs can be encoded by two or more sequences (*e.g.,* germline sequences) that are not contiguous (*e.g.,* in an unrearranged nucleic acid sequence) but are contiguous in a B cell nucleic acid sequence, *e.g.,* as a result of splicing or connecting the sequences (*e.g.,* V-D-J recombination to form a heavy chain CDR3).

[000505] The term "dissociative half-life" or "$t_{1/2}$" as used herein refers to the value calculated by the following formula: $t_{1/2}$ (min)=(ln2/k_d)/60, wherein k_d represents a dissociation rate constant.

[000506] The term "germline" in reference to an immunoglobulin nucleic acid sequence includes a nucleic acid sequence that can be passed to progeny.

[000507] The phrase "heavy chain," or "immunoglobulin heavy chain" includes an

immunoglobulin heavy chain sequence, including immunoglobulin heavy chain constant region sequence, from any organism. Heavy chain variable domains include three heavy chain CDRs and four FR regions, unless otherwise specified. Fragments of heavy chains include CDRs, CDRs and FRs, and combinations thereof. A typical heavy chain has, following the variable domain (from N-terminal to C-terminal), a C_H1 domain, a hinge, a C_H2 domain, and a C_H3 domain. A functional fragment of a heavy chain includes a fragment that is capable of specifically recognizing an epitope (e.g., recognizing the epitope with a K_D in the micromolar, nanomolar, or picomolar range), that is capable of expressing and secreting from a cell, and that comprises at least one CDR. Heavy chain variable domains are encoded by variable region nucleotide sequence, which generally comprises V_H, D_H, and J_H segments derived from a repertoire of V_H, D_H, and J_H segments present in the germline. Sequences, locations and nomenclature for V, D, and J heavy chain segments for various organisms can be found in IMGT database, which is accessible via the internet on the world wide web (www) at the URL “imgt.org.”

[000508] The phrase “light chain” includes an immunoglobulin light chain sequence from any organism, and unless otherwise specified includes human kappa (κ) and lambda (λ) light chains and a VpreB, as well as surrogate light chains. Light chain variable domains typically include three light chain CDRs and four framework (FR) regions, unless otherwise specified. Generally, a full-length light chain includes, from amino terminus to carboxyl terminus, a variable domain that includes FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, and a light chain constant region amino acid sequence. Light chain variable domains are encoded by the light chain variable region nucleotide sequence, which generally comprises light chain V_L and light chain J_L gene segments, derived from a repertoire of light chain V and J gene segments present in the germline. Sequences, locations and nomenclature for light chain V and J gene segments for various organisms can be found in IMGT database, which is accessible via the internet on the world wide web (www) at the URL “imgt.org.” Light chains include those, *e.g.*, that do not selectively bind either a first or a second epitope selectively bound by the epitope-binding protein in which they appear. Light chains also include those that bind and recognize, or assist the heavy chain with binding and recognizing, one or more epitopes selectively bound by the epitope-binding protein in which they appear.

[000509] The phrase “operably linked” refers to a relationship wherein the components operably linked function in their intended manner. In one instance, a nucleic acid sequence encoding a protein may be operably linked to regulatory sequences (e.g., promoter, enhancer, silencer sequence, etc.) so as to retain proper transcriptional regulation. In one instance, a nucleic acid sequence of an immunoglobulin variable region (or V(D)J segments)

may be operably linked to a nucleic acid sequence of an immunoglobulin constant region so as to allow proper recombination between the sequences into an immunoglobulin heavy or light chain sequence.

[000510] The phrase "somatically mutated," as used herein, includes reference to a nucleic acid sequence from a B cell that has undergone class-switching, wherein the nucleic acid sequence of an immunoglobulin variable region, *e.g.,* a heavy chain variable region (*e.g.,* a heavy chain variable domain or including a heavy chain CDR or FR sequence) in the class-switched B cell is not identical to the nucleic acid sequence in the B cell prior to class-switching, such as, for example a difference in a CDR or a framework nucleic acid sequence between a B cell that has not undergone class-switching and a B cell that has undergone class-switching. The phrase "somatically mutated" includes reference to nucleic acid sequences from affinity-matured B cells that are not identical to corresponding immunoglobulin variable region nucleotide sequences in B cells that are not affinity-matured (*i.e.,* sequences in the genome of germline cells). The phrase "somatically matured" also includes reference to an immunoglobulin variable region nucleic acid sequence from a B cell after exposure of the B cell to an epitope of interest, wherein the nucleic acid sequence differs from the corresponding nucleic acid sequence prior to exposure of the B cell to the epitope of interest. The term "somatically mutated" also refers to sequences from antibodies that have been generated in an animal, *e.g.,* a mouse having human immunoglobulin variable region nucleic acid sequences, in response to an immunogen challenge, and that result from the selection processes inherently operative in such an animal.

Non-Human Animals that Express Immunoglobulin Heavy Chain Variable Domain Comprising Histidine Residues

[000511] The described invention provides genetically modified non-human animals that can produce antigen-binding proteins with pH-dependent antigen binding characteristics. In various embodiments, the antigen-binding proteins produced by the genetically modified non-human animals as described herein exhibit increased pH-dependent recycling efficiency and/or enhanced serum half-life. In particular, the described invention employs genetic modifications in the immunoglobulin heavy chain locus to introduce histidine codons into a human heavy chain variable region nucleotide sequence and, optionally, to introduce a mutation(s) in a constant region nucleotide sequence that encodes C_H2 and/or C_H3 domains that increases the binding of the antibody constant region to an FcRn receptor, which facilitates recycling of the antigen-binding protein. Antigen-binding proteins comprising the modification may more loosely bind its target in an acidic intracellular compartment (*e.g.*, in an endosome where pH ranges from about 5.5 to about 6.0) than in an extracellular

environment or at the surface of a cell (*i.e.*, at a physiological pH, *e.g.*, a pH ranging from about 7.0 to about 7.4) due to protonated histidine residues located in the antigen binding sites. Therefore, the antigen-biding proteins comprising the genetic modifications as described herein would be able to be recycled more rapidly or efficiently than wild-type antigen-binding proteins that do not comprise such genetic modifications following target-mediated endocytosis. Furthermore, since the modified histidine residues are protonated only in an acidic environment, but not at a neutral pH, it is expected that such modification would not affect binding affinity and/or specificity of the antigen-binding protein toward an antigen of interest at a physiological pH.

[000512] In various aspects, non-human animals are provided comprising immunoglobulin heavy chain loci that comprise an unrearranged human heavy chain variable region nucleotide sequence, wherein the unrearranged human heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon.

[000513] In various aspects, methods of making and using the non-human animals are also provided. When immunized with an antigen of interest, the genetically modified non-human animals are capable of generating B cell populations that produce antigen-binding proteins comprising heavy chain variable domains with histidine residues, wherein the antigen-binding proteins exhibit enhanced pH-dependent recycling and/or increased serum half-life. In various embodiments, the non-human animals generate B cell populations that express human heavy chain variable domains along with cognate human light chain variable domains. In various embodiments, the genetically modified immunoglobulin heavy chain loci are present in a germline genome of the non-human animal.

[000514] In various embodiments, the genetically modified immunoglobulin heavy chain locus comprises a modification that deletes or renders, all or substantially all, non-functional endogenous V_H, D, and J_H gene segments; and the genetically modified locus comprises an unrearranged heavy chain variable region nucleotide sequence comprising one or more human V_H, D, and/or J_H gene segments having one or more histidine codons, wherein the unrearranged heavy chain variable region nucleotide sequence is present at an endogenous location (*i.e.,* where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.,* at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome). In one embodiment, *e.g.*, about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or

about 99% or more of all endogenous heavy chain V, D, or J gene segments are deleted or rendered non-functional. In one embodiment, *e.g.,* at least 95%, 96%, 97%, 98%, or 99% of endogenous functional heavy chain V, D, or J gene segments are deleted or rendered non-functional.

[000515] In one embodiment, the non-human animal is a mammal. Although embodiments directed to introducing histidine codons into an unrearranged human heavy chain variable gene sequence in a mouse are extensively discussed herein, other non-human animals are also provided that comprise a genetically modified immunoglobulin locus containing an unrearranged human heavy chain variable region nucleotide sequence comprising an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon. Such non-human animals include any of those which can be genetically modified to express the histidine-containing heavy chain variable domain as disclosed herein, including, *e.g.*, mouse, rat, rabbit, pig, bovine (*e.g.*, cow, bull, buffalo), deer, sheep, goat, chicken, cat, dog, ferret, primate (*e.g.*, marmoset, rhesus monkey), etc. For example, for those non-human animals for which suitable genetically modifiable ES cells are not readily available, other methods are employed to make a non-human animal comprising the genetic modification. Such methods include, *e.g.*, modifying a non-ES cell genome (*e.g.*, a fibroblast or an induced pluripotent cell) and employing somatic cell nuclear transfer (SCNT) to transfer the genetically modified genome to a suitable cell, *e.g.,* an enucleated oocyte, and gestating the modified cell (*e.g.*, the modified oocyte) in a non-human animal under suitable conditions to form an embryo. Methods for modifying a non-human animal genome (*e.g.*, a pig, cow, rodent, chicken, etc. genome) include, *e.g.*, employing a zinc finger nuclease (ZFN) or a transcription activator-like effector nuclease (TALEN) to modify a genome to include a nucleotides sequence that encodes

[000516] In one embodiment, the non-human animal is a small mammal, *e.g.*, of the superfamily *Dipodoidea* or *Muroidea*. In one embodiment, the genetically modified animal is a rodent. In one embodiment, the rodent is selected from a mouse, a rat, and a hamster. In one embodiment, the rodent is selected from the superfamily *Muroidea*. In one embodiment, the genetically modified animal is from a family selected from *Calomyscidae* (*e.g.*, mouse-like hamsters), *Cricetidae* (*e.g.*, hamster, New World rats and mice, voles), *Muridae* (true mice and rats, gerbils, spiny mice, crested rats), *Nesomyidae* (climbing mice, rock mice, with-tailed rats, Malagasy rats and mice), *Platacanthomyidae* (*e.g.*, spiny dormice), and *Spalacidae* (*e.g.*, mole rates, bamboo rats, and zokors). In a specific embodiment, the genetically modified rodent is selected from a true mouse or rat (family *Muridae*), a gerbil, a

spiny mouse, and a crested rat. In one embodiment, the genetically modified mouse is from a member of the family *Muridae*. In one embodiment, the animal is a rodent. In a specific embodiment, the rodent is selected from a mouse and a rat. In one embodiment, the non-human animal is a mouse.

[000517] In one embodiment, the non-human animal is a rodent that is a mouse of a C57BL strain selected from C57BL/A, C57BL/An, C57BL/GrFa, C57BL/KaLwN, C57BL/6, C57BL/6J, C57BL/6ByJ, C57BL/6N, C57BL/6NJ, C57BL/10, C57BL/10ScSn, C57BL/10Cr, and C57BL/Ola. In another embodiment, the mouse is a 129 strain. In one embodiment, the 129 strain is selected from the group consisting of 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 one embodiment, the genetically modified mouse is a mix of an aforementioned 129 strain and an aforementioned C57BL strain (*e.g.*, a C57BL/6 strain). In another embodiment, the mouse is a mix of aforementioned 129 strains, or a mix of aforementioned C57BL/6 strains. In one embodiment, the 129 strain of the mix is a 129S6 (129/SvEvTac) strain. In another embodiment, the mouse is a mix of a 129/SvEv- and a C57BL/6-derived strain. In a specific embodiment, the mouse is a mix of a 129/SvEv- and a C57BL/6-derived strain as described in Auerbach *et al.* 2000 *BioTechniques* 29:1024-1032. In another embodiment, the mouse is a BALB strain, *e.g.,* BALB/c strain. In another embodiment, the mouse is a mix of a BALB strain (*e.g.,* BALB/c strain) and another aforementioned strain.

[000518] In one embodiment, the non-human animal is a rat. In one embodiment, the rat is selected from a Wistar rat, an LEA strain, a Sprague Dawley strain, a Fischer strain, F344, F6, and Dark Agouti. In one embodiment, the rat strain is a mix of two or more of a strain selected from the group consisting of Wistar, LEA, Sprague Dawley, Fischer, F344, F6, and Dark Agouti.

[000519] In one embodiment, the non-human animal is a mouse. In one embodiment, the mouse is a VELOCIMMUNE® humanized mouse.

[000520] VELOCIMMUNE® humanized mice (see, *e.g.,* US 6,596,541, US 7,105,348, and US20120322108A1, which are incorporated herein by reference in their entireties), which contain a precise replacement of mouse immunoglobulin variable regions with human immunoglobulin variable regions at the endogenous mouse loci, display a surprising and

remarkable similarity to wild-type mice with respect to B cell development. VELOCIMMUNE® humanized mice display an essentially normal, wild-type response to immunization that differed only in one significant respect from wild-type mice - the variable regions generated in response to immunization are fully human.

[000521] VELOCIMMUNE® humanized mice contain a precise, large-scale replacement of germline variable region nucleotide sequences of mouse immunoglobulin heavy chain (IgH) and immunoglobulin light chain (e.g., κ light chain, Igκ) with corresponding human immunoglobulin variable region nucleotide sequences, at the endogenous loci (see, *e.g.,* US 6,596,541, US 7,105,348, US 20120322108A1, which are incorporated herein by reference in their entireties). In total, about six megabases of mouse loci are replaced with about 1.5 megabases of human genomic sequence. This precise replacement results in a mouse with hybrid immunoglobulin loci that make heavy and light chains that have a human variable regions and a mouse constant region. The precise replacement of mouse V_H-D-J_H and Vκ-Jκ segments leave flanking mouse sequences intact and functional at the hybrid immunoglobulin loci. The humoral immune system of the mouse functions like that of a wild-type mouse. B cell development is unhindered in any significant respect and a rich diversity of human variable regions is generated in the mouse upon antigen challenge.

[000522] VELOCIMMUNE® humanized mice are possible because immunoglobulin gene segments for heavy and κ light chains rearrange similarly in humans and mice, which is not to say that their loci are the same or even nearly so—clearly they are not. However, the loci are similar enough that humanization of the heavy chain variable gene locus can be accomplished by replacing about three million base pairs of contiguous mouse sequence that contains all the V_H, D, and J_H gene segments with about one million bases of contiguous human genomic sequence covering basically the equivalent sequence from a human immunoglobulin locus.

[000523] In some embodiments, further replacement of certain mouse constant region nucleotide sequences with human constant region nucleotide sequences (*e.g.*, replacement of mouse heavy chain C_H1 nucleotide sequence with human heavy chain C_H1 nucleotide sequence, and replacement of mouse light chain constant region nucleotide sequence with human light chain constant region nucleotide sequence) results in mice with hybrid immunoglobulin loci that make antibodies that have human variable regions and partly human constant regions, suitable for, *e.g.*, making fully human antibody fragments, *e.g.*, fully human Fab's. Mice with hybrid immunoglobulin loci exhibit normal variable gene segment rearrangement, normal somatic hypermutation frequencies, and normal class switching. These mice exhibit a humoral immune system that is indistinguishable from wild type mice,

and display normal cell populations at all stages of B cell development and normal lymphoid organ structures—even where the mice lack a full repertoire of human variable region nucleotide segments. Immunizing these mice results in robust humoral responses that display a wide diversity of variable gene segment usage.

[000524] The precise replacement of the mouse germline variable region nucleotide sequence allows for making mice that have partly human immunoglobulin loci. Because the partly human immunoglobulin loci rearrange, hypermutate, and class switch normally, the partly human immunoglobulin loci generate antibodies in a mouse that comprise human variable regions. Nucleotide sequences that encode the variable regions can be identified and cloned, then fused (*e.g.*, in an *in vitro* system) with any sequences of choice, *e.g.*, any immunoglobulin isotype suitable for a particular use, resulting in an antibody or antigen-binding protein derived wholly from human sequences.

[000525] In various embodiments, at least one histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes an N-terminal region, a loop 4 region, a CDR1, a CDR2, a CDR3, or a combination thereof.

[000526] In various embodiments, at least one histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes a framework region (FR) selected from the group consisting of FR1, FR2, FR3, and FR4.

[000527] In various aspects, the genetically modified immunoglobulin locus comprises a nucleotide sequence wherein at least one codon has been replaced with a histidine codon.

[000528] In various aspects, the genetically modified immunoglobulin locus comprises an unrearranged human heavy chain variable region nucleotide sequence comprising a substitution of at least one endogenous non-histidine codon with a histidine codon.

[000529] In one embodiment, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, 40 or more, 41 or more, 42 or more, 43 or more, 44 or more, 45 or more, 46 or more, 47 or more, 48 or more, 49 or more, 50 or more, 51 or more, 52 or more, 53 or more, 54 or more, 55 or more, 56 or more, 57 or more, 58 or more, 59 or more, 60 or more, or 61 or more of the endogenous non-histidine codons are replaced with histidine codons.

[000530] Previous studies on reading frame usage of human immunoglobulin D gene segments have shown that, of the three reading frames (*i.e.,* stop, hydrophobic, and hydrophilic), the stop frame is used very infrequently. Apparently, some stop frames are chewed back and result in expression. However, stop reading frames are used at such a low frequency that for the purposes of engineering histidine codons, it is more efficient not to use the stop reading frame. As between hydrophilic and hydrophobic reading frames, the hydrophilic reading frame appears to be preferred. Thus, in one embodiment, the hydrophilic reading frame of human D gene segments is engineered to contain one or more histidine codons (as compared with the stop frame or with the hydrophobic frame).

[000531] Methods of introducing a mutation *in vitro*, *e.g.*, site-directed mutagenesis, are well known in the art. In some embodiments of the described invention, histidine codons are enriched by designing histidine-substituted human D gene segments *in silico* (*e.g.,* mutation of Y, D, and N codons to H codons, *e.g.*, CAT, CAC), which are synthesized (*e.g.,* chemical synthesis) with (unique) restriction enzyme sites for ligating them back together. The synthesized D gene segments are made with the appropriate recombination signal sequences (RSS) upstream and downstream. In one embodiment, when ligated to one another, the synthesized histidine-substituted D gene segments include the intergenic sequences observed in a human between each D gene segment.

[000532] It is understood that the codons that encode the one or more histidines, upon rearrangement and/or somatic hypermutation, may change such that one or more of the histidines will be changed to another amino acid. However, this may not occur for each and every codon encoding histidine, in each and every rearrangement in the non-human animal. If such changes occur, the changes may occur in some but not all B cells or in some but not all heavy chain variable sequences.

[000533] In various aspects, the genetically modified immunoglobulin locus comprises a human heavy chain V, D, and J gene segment, wherein at least one of the human D gene segment has been inverted 5' to 3' with respect to a corresponding wild-type sequence, and wherein at least one reading frame of the inverted human D gene segment comprises a histidine codon.

[000534] In various embodiments, the nucleotide sequence comprises one or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, or 25 or more of histidine codons.

[000535] There are 25 functional human D gene segments in 6 families of 3-5 members each (one family- the D7 family- has a single member). Direct recombination of human D gene segments is much more frequent than inversion, although inverted reading frames exhibit more histidine codons. Certain D gene segments and reading frames are used more frequently than others. All three direct reading frames and all three inverted orientation reading frames for all the functional D gene segments are presented in Figs. 10A-10E. As shown in Figs. 10A-10E, there are many more histidine codons in inverted reading frames than in direct reading frames. More specifically, there are 34 histidines in inverted reading frames and only four in direct reading frames. In addition, of the four in direct reading frames, three histidines are encoded by pseudogenes or present in alternate alleles. Therefore, there is only a single direct reading frame of a germline human D gene segment that contains a histidine codon, with further histidine codons possibly encountered in alternate alleles (presumably in subsets of the human population).

[000536] Inverted D rearrangements are extremely rare. Tuaillon et al. (J. Immunol., 154(12): 5453-6465, incorporated by reference herein in its entirety) showed that usage of inverted reading frames (as measured by limiting dilution PCT) is very rare, *i.e.*, that the ratio of direct to indirect rearrangements are, in most cases, 100 to 1000. To the extent that the ratio of direct to indirect rearrangement was low, it was only observed in those D segments that exhibit very low usage. It was also shown that D gene segment family 7, which is located adjacent to J1 (far down from other D family members) is mostly used in fetuses, but exhibits a low usage in adults (Schroeder et al., Immunology 30, 2006, 119-135, incorporated by reference herein in its entirety). Therefore, in one embodiment, D family 7 sequences are not inverted 5' to 3'.

[000537] In one embodiment, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty one, at least twenty two, at least twenty three, at least twenty four, or all or substantially all of the human functional D gene segments are inverted 5' to 3' with respect to corresponding wild type sequences.

[000538] In one embodiment, the human immunoglobulin heavy chain variable domain comprising at least one non-naturally occurring histidine residue exhibits pH-dependent antigen binding characteristics. For example, an antibody comprising the modified immunoglobulin heavy chain variable domain binds a target with sufficient affinity at around a neutral pH (*e.g.*, pH of about 7.0 to about 7.4), but either does not bind or binds weaker to the same target at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0). In one embodiment, the

acidic pH is selected from about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, and about 6.0. In one embodiment, the neutral pH is selected from about 7.0, about 7.1, about 7.2, about 7.3, and about 7.4.

[000539] In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 25°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) at 37°C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (*e.g.,* pH of about 7.0 to about 7.4).

[000540] In one embodiment, antigen binding proteins comprising the genetically modified human immunoglobulin heavy chain variable domain is capable of specifically binding an antigen of interest with an affinity (K_D) lower than 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} or 10^{-10}, 10^{-11}, 10^{-12} at a neutral or physiological pH (pH of about 7.0 to about 7.4).

[000541] The altered binding property of the immunoglobulin heavy chain variable domain at an acidic pH (*e.g.,* pH of about 5.5 to about 6.0) would, in some circumstances, allow faster turnover of the antibody because the therapeutic antibody will bind a target on a cell's surface, be internalized into an endosome, and more readily or more rapidly dissociate from the target in the endosome, so that the therapeutic can be recycled to bind yet another molecule of target present in another cell. This would allow one to administer a therapeutic antibody at a lower dose, or administer the therapeutic antibody less frequently. This is

particularly useful in a situation where it is not desirable to administer a therapeutic antibody frequently, or administer at a level above a certain dosage for safety or toxicity reasons.

[000542] In various embodiments, the human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a human or non-human heavy chain constant region nucleotide sequence (*e.g.*, a heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA). In various embodiments, the human or non-human heavy chain constant region nucleotide sequence is selected from the group consisting of a C_H1, a hinge, a C_{H2}, a C_{H3}, and a combination thereof. In one embodiment, the constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (*e.g.*, C_H1-hinge-a C_H2-C_H3).

[000543] In various embodiments, the heavy chain constant region nucleotide sequence is present at an endogenous locus (*i.e.*, where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (*e.g.*, at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, *e.g.*, within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

[000544] In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000545] The neonatal Fc receptor for IgG (FcRn) has been well characterized in the transfer of passive humoral immunity from a mother to her fetus across the placenta and proximal small intestine (Roopenian, D. and Akilesh, S., Nat. Rev. Immun., 2007, 7:715-725, which is incorporated by reference herein in its entirety). FcRn binds to the Fc portion of IgG at a site that is distinct from the binding sites of the classical FcγRs or the C1q component of complement, which initiates the classical pathway of complement activation. More specifically, it was shown that FcRn binds the C_H2-C_H3 hinge region of IgG antibodies - a versatile region of Fc that also binds *Staphylococcal* protein A, *Streptococcal* protein G, and the rheumatoid factor. In contrast to other Fc-binding proteins, however, FcRn binds the Fc region of IgG in a strictly pH-dependent manner; at physiological pH 7.4, FcRn does not bind IgG, whereas at the acidic pH of the endosome (*e.g.*, where the pH ranges from about 5.5 to about 6.0), FcRn exhibits a low micromolar to nanomolar affinity for the Fc region of IgG. This pH-dependent interaction has been shown to be mediated by the titration of histidine residues in the C_H2-C_H3 region of IgG and their subsequent interaction with acidic residue on

the surface of FcRn (Roopenian, D. and Akilesh, S., Nat. Rev. Immun., 2007, 7:715-725, incorporated by reference in its entirety).

[000546] Various mutations in the C_H2-C_H3 region of IgG that can increase the affinity of Fc region to FcRn at an acidic pH are known in the art. These include, but are not limited to, modification at position 250 (*e.g.,* E or Q); 250 and 428 (*e.g.,* L or F); 252 (*e.g.,* L/Y/F/W or T), 254 (*e.g.,* S or T), and 256 (*e.g.,* S/R/Q/E/D or T); or a modification at 428 and/or 433 (*e.g.,* L/R/S/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at 250 and/or 428; or a modification at 307 or 308 (*e.g.,* 308F, V308F), and 434. In another example, the modification can comprise a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 52Y, 254T, and 256E) modification; a 250Q and 428L modification, or a combination thereof.

[000547] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000548] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000549] In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0).

[000550] In one embodiment, the human constant region amino acid sequence encoded by the heavy chain constant region nucleotide sequence described herein comprises a mutation selected from the group consisting of M428L, N434S, and a combination thereof. In one embodiment, the human constant region amino acid sequence comprises a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof. In one embodiment, the human constant region amino acid sequence comprises an N434A

mutation. In one embodiment, the human constant region amino acid sequence comprises a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof. In one embodiment, the human constant region amino acid sequence comprises a mutation selected from the group consisting of T250Q, M248L, or both. In one embodiment, the human constant region amino acid sequence comprises a mutation selected from the group consisting of H433K, N434Y, or both.

[000551] In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

[000552] In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

EXAMPLES

[000553] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Construction of Humanized Immunoglobulin Heavy Chain Loci Comprising Histidine-Substituted D Gene Segments

[000554] Construction of immunoglobulin heavy chain loci comprising histidine-substituted human D gene segments was carried out by series of homologous recombination reactions in bacterial cells (BHR) using Bacterial Artificial Chromosome (BAC) DNA. Several targeting constructs for creation of a genetically engineered mouse that expresses a heavy chain variable domain comprising one or more histidine residues were generated using VELOCIGENE® genetic engineering technology (see, *e.g.*, US Pat. No. 6,586,251 and Valenzuela, D. M. *et al.* (2003), High-throughput engineering of the mouse genome coupled

with high-resolution expression analysis, *Nature Biotechnology* 21(6):652-659, which is incorporated herein by reference in their entireties).

[000555] Initially, human D gene segments were synthesized *in silico* as four pieces (4 repeats) in which the codons encoding tyrosine (Y), asparagine (N), serine (S), glycine (G), and aspartate (D) in the hydrophilic frame were substituted with histidine codons (hereinafter "histidine-substituted human D gene segments", *i.e.*, HD 1.1-6.6 (9586bp; SEQ ID NO: 1), HD 1.7-6.13 (9268bp; SEQ ID NO: 2), HD 1.14-6.19 (9441bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592bp; SEQ ID NO: 4) (Fig. 3). The four repeats also contained unique restriction enzyme sites at the ends for ligating them back together. The specific location of the histidine substitutions (labeled in bold type) in each human D gene segment is shown in Figs. 1A and 1B in the column labeled "Hydrophilic." As shown in Fig. 1, while the modification introduced histidine codons in the hydrophilic reading frame, it also changed some stop codons to serine codons in the "Stop" reading frame. The modification, however, made few changes in the "Hydrophobic" reading frame. The detailed procedure for ligating the four synthesized D segment repeats is illustrated in Fig. 3 (sequential ligation). The resulting clone contained, from 5' to 3', a 5' mouse homology arm, a floxed neomycin cassette, human D gene segments comprising histidine substitutions (*i.e.*, HD 1.1-6.6 (9586bp; SEQ ID NO: 1), HD 1.7-6.13 (9268bp; SEQ ID NO: 2), HD 1.14-6.19 (9441bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592bp; SEQ ID NO: 4)), a chloramphenicol selection cassette, and a 3' homology arm.

[000556] The following six genetic modifications were carried out in order to replace the endogenous human D gene segments in the VELOCIMMUNE® humanized mouse with the histidine-substituted human D gene segments described above.

[000557] First, pLMa0174, containing a spectinomycin selection cassette and an AsiSI restriction site, was targeted into the 5' end of the MAID 1116 clone (Step 1. BHR (Spec); Fig. 2). During Step 1, a chloramphenicol selection cassette, a neomycin selection cassette, a loxP site, two V_H gene segments (hV_H1-3 and hV_H1-2), and the human Adam6p gene, all of which are located 5' upstream of hV_H6-1, were deleted from the MAID 1116 clone and replaced by a spectinomycin cassette to yield the VI433 clone.

[000558] Second, in Step 2 (BHR (Hyg + Spec); Fig. 2), pNTu0002 containing a hygromycin cassette flanked by FRT sites was targeted into a region comprising human immunoglobulin D_H gene segments. During Step 2, all human heavy chain D gene segments were deleted from VI433 and replaced with the hygromycin cassette to yield MAID6011 VI 434 (clone 1). The modification also introduced the PI-SceI and the I-CeuI restriction sites at

the 5' and 3' end of the hygromycin cassette.

[000559] Third, the genomic region comprising histidine-substituted human D gene segments (HD 1.1-6.6 (9586bp; SEQ ID NO: 1), HD 1.7-6.13 (9268bp; SEQ ID NO: 2), HD 1.14-6.19 (9441bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592bp; SEQ ID NO: 4)) were introduced into a region between the PI-SceI and the I-CeuI sites of MAID 6011 VI434 via restriction digestion and ligation (PI-SceI/I-CeuI Ligation modified 1116 (Kan + Spec); Fig. 4). This yielded MAID6012 VI469 containing, from 5' to 3', a spectinomycin cassette, about 50 kb of a genomic region comprising V_H6-1, a floxed neomycin cassette, about 40 kb of the histidine-substituted human D gene segments (HD 1.1-6.6 (9586bp; SEQ ID NO: 1), HD 1.7-6.13 (9268bp; SEQ ID NO: 2), HD 1.14-6.19 (9441bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592bp; SEQ ID NO: 4)), and about 25 kb of a genomic region containing human J_H gene segments, followed by a mouse E_i (mIgH intronic enhancer; SEQ ID NO: 5), a mouse switch region (SEQ ID NO: 6), and a mouse IgM constant region nucleotide sequence (mIgM exon 1; SEQ ID NO: 7). Bacterial cells containing the modification were selected based on Kanamycin and Spectinomycin selection.

[000560] Fourth, MAID 1460 heterozygous mouse ES cells were targeted with MAID 6011 VI434 via electroporation in order to remove all endogenous human D gene segments from the MAID 1460 clone as illustrated in Fig. 5. This yielded MAID 6011 heterozygous mouse ES cells comprising in its immunoglobulin heavy chain locus (at the 129 strain-derived chromosome), from 5' to 3', an FRT site, human V_H gene segments, a mouse genomic region encompassing adam6a/b genes, a hygromycin cassette flanked by FRT sites, and human J_H segments, followed by a mouse E_i sequence and an IgM constant region nucleotide sequence. The genetic modification of MAID 6011 (a loss of alleles, a gain of alleles, and presence of parental alleles) was confirmed by using the probes and primers as shown in Fig 6.

[000561] Fifth, MAID 6011 heterozygous mouse ES cells were electroporated with MAID 6012 VI469 in order to introduce histidine-substituted human D gene segments (*i.e.*, HD 1.1-6.6 (9586bp; SEQ ID NO: 1), HD 1.7-6.13 (9268bp; SEQ ID NO: 2), HD 1.14-6.19 (9441bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592bp; SEQ ID NO: 4)) into MAID 6011. The targeting step removed the floxed hygromycin selection cassette from MAID 6011 and replaced the sequence with the histidine-substituted human D gene segments. This lead to MAID 6012 hetrozygous ES cells comprising a wild-type C57BL/6 strain-derived chromosome and a genetically modified 129 strain-derived chromosome comprising human wild-type V_H and J_H gene segments and the histidine-substituted human D gene segments described herein. In addition, the ES cells contained a mouse genomic region encompassing

adam6a/b genes and a floxed neomycin cassette between the V_H and D segments (Fig. 7). The genetic modification of MAID 6012 (a loss of alleles, a gain of alleles, and presence of parental alleles) was confirmed by using the probes and primers as shown in Fig 8.

[000562] Lastly, MAID 6012 ES cells were electroporated with a plasmid that expresses a Cre recombinase in order to remove the neomycin selection cassette from the MAID 6012 ES cells, resulting in MAID 6013 heterozygous ES cells (Fig. 9). The final MAID 6013 heterozygous ("MAID 6013 het") ES cell contains a wild-type C57BL/6 strain-derived chromosome and a genetically modified, 129 strain-derived chromosome comprising in its immunoglobulin heavy chain locus, from 5' to 3', (1) an FRT site; (2) human V_H gene segments; (3) a mouse genomic region encompassing adam6a/b genes; (4) a floxed neomycin selection cassette; (5) histidine-substituted human D gene segments (HD 1.1-6.6 (9586bp; SEQ ID NO: 1), HD 1.7-6.13 (9268bp; SEQ ID NO: 2), HD 1.14-6.19 (9441bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592bp; SEQ ID NO: 4)); (6) human J_H gene segments; followed by (7) a mouse E_i sequence (mIgH intronic enhancer; SEQ ID NO: 5), (8) a switch region (SEQ ID NO: 6); and (9) a mouse IgM constant region nucleotide sequence (mIgM exon 1; SEQ ID NO: 7) as illustrated in Fig. 9.

[000563] The targeted ES cells (MAID 6013) described above were used as donor ES cells and introduced into an 8-cell stage mouse embryo by the VELOCIMOUSE® method (see, *e.g.*, US 7,576,259, US 7,659,442, US 7,294,754, US 2008-0078000 A1, all of which are incorporated by reference herein in their entireties). Mice bearing the genetically modified immunoglobulin heavy chain locus comprising the histidine-substituted human heavy chain D gene segments described herein were identified by genotyping using the primers and probes set forth in Fig. 8. The resulting genetically modified F0 mouse was crossed to a wild-type mouse to obtain F1 offspring. F1 pups were genotyped, and the F1 pups that are heterozygous for the genetically modified immunoglobulin locus comprising histidine-substituted human heavy chain D gene segments were selected for further characterization.

Example 2. Analysis of Rearranged Heavy Chain Variable Region Nucleotide Sequences

[000564] Next, it was examined whether the genetically modified mouse comprising histidine-substituted human D gene segments described herein, *i.e.*, 6013 F0 heterozygous mouse, which comprises in its germline a 129 strain-derived chromosome comprising human V_H, J_H gene segments, and histidine-substituted human D gene segments (HD 1.1-6.6 (9586bp; SEQ ID NO: 1), HD 1.7-6.13 (9268bp; SEQ ID NO: 2), HD 1.14-6.19 (9441bp;

SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592bp; SEQ ID NO: 4), can express rearranged heavy chain V(D)J sequences comprising one or more histidine codons derived from the genetically modified immunoglobulin heavy chain locus.

[000565] To this end, mRNA sequences encoding IgM heavy chain variable region were analyzed for the presence of IgM CDR3 sequences derived from the histidine-substituted human D gene segments via high throughput sequencing. Briefly, spleens were harvested and homogenized in 1xPBS (Gibco) using glass slides. Cells were pelleted in a centrifuge (500xg for 5 minutes), and red blood cells were lysed in ACK Lysis buffer (Gibco) for 3 minutes. Cells were washed with 1xPBS and filtered using a 0.7μm cell strainer. B-cells were isolated from spleen cells using MACS magnetic positive selection for CD19 (Miltenyi Biotec). Total RNA was isolated from pelleted B-cells using the RNeasy Plus kit(Qiagen). PolyA+ mRNA was isolated from total RNA using the Oligotex® Direct mRNA mini kit (Qiagen).

[000566] Double-stranded cDNA was prepared from splenic B cell mRNA by 5' RACE using the SMARTer™ Pico cDNA Synthesis Kit (Clontech). The Clontech reverse transcriptase and dNTPs were substituted with Superscript II and dNTPs from Invitrogen. Heavy chain variable region (V_H) antibody repertoires were amplified from the cDNA using primers specific for IgM constant regions and the SMARTer™ 5' RACE primer (Table 1). PCR products were cleaned up using a QIAquick® PCR Purification Kit (Qiagen). A second round of PCR was done using the same 5' RACE primer and a nested 3' primer specific for the IgM constant regions (Table 2). The second round PCR products were purified using a SizeSelect™ E-gel® system (Invitrogen). A third PCR was performed with primers that added 454 adapters and barcodes. The third round PCR products were purified using Agencourt® AMPure® XP Beads. Purified PCR products were quantified by SYBR®-qPCR using a KAPA Library Quantification Kit (KAPA Biosystems). Pooled libraries were subjected to emulsion PCR (emPCR) using the 454 GS Junior Titanium Series Lib-A emPCR Kit (Roche Diagnostics) and bidirectional sequencing using Roche 454 GS Junior instrument according to the manufacturer's protocols.

[000567] Table 1.

NAME	SEQUENCE
3' mIgM CH1 outer	TCTTATCAGACAGGGGGCTCTC (SEQ ID NO:321)

[000568] Table 2.

NAME	SEQUENCE
3' mIgM CH1 inner	GGAAGACATTTGGGAAGGACTG (SEQ ID NO:322)

[000569] Bioinfomatic Analysis

[000570] The 454 sequences were sorted based on the sample barcode perfect match and trimmed for quality. Custom D database was created using histidine-substituted human D-gene segments. Sequences were annotated based on alignment of rearranged Ig sequences to human germline V and J gene segments database using local installation of igblast (NCBI, v2.2.25+). Sequences derived from the endogenous mouse immunoglobulin heavy chain locus were filtered out using similarity threshold of 90%. A sequence was marked as ambiguous and removed from analysis when multiple best hits with identical score were detected. A set of perl scripts was developed to analyze results and store data in mysql database. The CDR3 region was defined between conserved C codon and FGXG motif (SEQ ID NO: 323) for light chains and WGXG motif (SEQ ID NO: 324) for heavy chains. CDR3 length was determined using only productive antibodies. Number of histidine codons was calculated for each CDR3 region.

[000571] As shown in Figs. 11-13, the 6013 F0 heterozygous mice expressed a diverse repertoire of rearranged heavy chain variable region mRNA sequences (rearranged V-D-J sequences) encoding one or more histidine codons in CDR3. The sequencing and alignment data suggested that the histidine codons appeared in CDR3 sequences were derived from various histidine-substituted human D gene segments present in the genetically modified immunoglobulin heavy chain locus of the 6013 mice described herein. In addition, as compared with control mice comprising human V_H, D_H, J_H gene segments and mouse adam6 genes (VI3-Adam6, US Publication No. 2012/0322108A1, which is incorporated by reference in its entirety), the genetically modified 6013 F0 heterozygous mice exhibited a higher frequency of histidine occurrence in the heavy chain CDR3 sequences (Fig. 14).

[000572] While the described invention has been described with reference to the specific embodiments thereof it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adopt a particular situation, material, composition of matter, process, process step or steps, to the objective spirit and scope of the described invention. All such modifications are intended to be within the scope of the claims appended hereto.

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.