HISTIDINE ENGINEERED LIGHT CHAIN ANTIBODIES AND GENETICALLY MODIFIED NON-HUMAN ANIMALS FOR GENERATING THE SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit under 35 U.S.C. §119(e) of U.S. Provisional Application No. 61/611,950, filed March 16, 2012, and U.S. Provisional Application No. 61/736,930, filed December 13, 2012, both incorporated by reference herein in their entireties.

FIELD OF INVENTION

[0002] A genetically modified non-human animal (e.g., rodent, e.g., mouse or rat) is provided that expresses antibodies capable of binding to an antigen in a pH dependent manner. A method for making modifications to immunoglobulin light chain variable region sequence of a non-human animal is provided, wherein the modifications include the mutagenesis of residues within the light chain variable region gene, *e.g.,* nucleotides that encode one or more amino acids within a complementary determining region (CDR), to facilitate *in vivo* expression of antibodies comprising light chain domains that exhibit pH dependent binding to antigens. Methods for making antibodies with pH-dependent antigen binding are also provided.

BACKGROUND OF THE INVENTION

[0003] Antibodies typically comprise a homodimeric heavy chain component, wherein each heavy chain monomer is associated with an identical light chain. Antibodies having a heterodimeric heavy chain component (e.g., bispecific antibodies) are desirable as therapeutic antibodies. But making bispecific antibodies having a suitable light chain component that can satisfactorily associate with each of the heavy chains of a bispecific antibody has proved problematic.

[0004] In one approach, a light chain might be selected by surveying usage statistics for all light chain variable domains, identifying the most frequently employed light chain in human antibodies, and pairing that light chain *in vitro* with the two heavy chains of differing specificity.

[0005] In another approach, a light chain might be selected by observing light chain sequences in a phage display library (e.g., a phage display library comprising human light chain variable region sequences, e.g., a human scFv library) and selecting the most commonly used light chain variable region from the library. The light chain can then be tested on the two different heavy chains of interest.

[0006] In another approach, a light chain might be selected by assaying a phage display library of light chain variable sequences using the heavy chain variable sequences of both heavy chains of interest as probes. A light chain that associates with both heavy chain variable sequences might be selected as a light chain for the heavy chains.

[0007] In another approach, a candidate light chain might be aligned with the heavy chains' cognate light chains, and modifications are made in the light chain to more closely match sequence characteristics common to the cognate light chains of both heavy chains. If the chances of immunogenicity need to be minimized, the modifications preferably result in sequences that are present in known human light chain sequences, such that proteolytic processing is unlikely to generate a T cell epitope based on parameters and methods known in the art for assessing the likelihood of immunogenicity (i.e., *in silico* as well as wet assays).

[0008] All of the above approaches rely on *in vitro* methods that subsume a number of *a priori* restraints, e.g., sequence identity, ability to associate with specific pre-selected heavy chains, etc. There is a need in the art for compositions and methods that do not rely on manipulating *in vitro* conditions, but that instead employ more biologically sensible approaches to making human epitope-binding proteins that include a common light chain.

[0009] In addition, therapeutic antibodies, e.g., bispecific therapeutic antibodies, have some limitations in that they often require high doses to achieve desired efficacy. This is partly due to the fact that antibody-antigen complexes are internalized into the endosome, and are targeted for lysosomal degradation in a process called target-mediated clearance. Thus, there is a need in the art for methods and compositions that lead to more efficient antibody recycling, e.g., bispecific antibody recycling, and prevent degradation of the antibody by promoting dissociation of antibody-antigen complexes in the endosomal compartment without compromising the specificity and affinity of the antibody toward the antigen.

SUMMARY OF THE INVENTION

[0010] In one aspect, a biological system is provided for generating an antibody or an antibody variable domain that binds a target antigen at a neutral pH but exhibits reduced binding of the same antigen at an acidic pH (*e.g.*, pH 5.0-6.0). The biological system comprises a non-human animal, *e.g.*, a rodent (*e.g,* a mouse or rat) that has a rearranged light chain sequence (*e.g.*, a rearranged V-J) that comprises one or more histidine modifications. In various aspects, the one or more histidine modifications are in the light chain CDR3 codon. In various aspects, the non-human animal comprises a human or humanized heavy chain immunoglobulin locus. In various aspects, the non-human animal comprises a replacement of endogenous non-human heavy chain variable gene segments

with one or more human heavy chain V_H, D_H, and J_H segments, wherein the human segments are operably linked to a non-human immunoglobulin constant region. In various aspects, non-human animals with universal light chains comprising light chain variable domains with substitutions of non-histidine residues for histidine residues are provided. In various aspects these histidine-modified universal light chain non-human animals (*e.g.,* rodents, *e.g.,* mice) are referred to as histidine-universal light chain mice, histidine-ULC mice, or HULC mice.

[0011] Thus, in one aspect, provided herein is a genetically modified non-human animal that comprises in its germline an immunoglobulin light chain locus that comprises a single rearranged human immunoglobulin light chain variable region gene sequence comprising human V_L and J_L segment sequences, wherein the single rearranged human immunoglobulin light chain variable region sequence comprises a substitution of at least one non-histidine codon with a histidine codon. In one embodiment, the single rearranged human immunoglobulin variable region sequence is operably linked to an immunoglobulin light chain constant region gene sequence. In one embodiment, the immunoglobulin light chain constant region gene sequence is a non-human immunoglobulin light chain constant region gene sequence. In one embodiment, the non-human immunoglobulin light chain constant region gene sequence is an endogenous immunoglobulin light chain constant region gene sequence. In one embodiment, the non-human animal lacks a functional unrearranged immunoglobulin light chain variable region. In one embodiment, the immunoglobulin light chain locus is at an endogenous non-human immunoglobulin light chain locus.

[0012] In one embodiment, the animal further comprises in its germline an immunoglobulin heavy chain locus that comprises an unrearranged immunoglobulin heavy chain variable region gene sequence comprising human V_H, D_H, and J_H segments operably linked to an immunoglobulin heavy chain constant region gene sequence. In one embodiment, the immunoglobulin heavy chain constant region gene sequence is a non-human heavy chain constant region gene sequence. In one embodiment, the non-human heavy chain constant region gene sequence is an endogenous immunoglobulin heavy chain constant region gene sequence. In one embodiment, the immunoglobulin heavy chain locus is at an endogenous immunoglobulin heavy chain locus.

[0013] In one embodiment, the substitution of at least one non-histidine codon with a histidine codon is in the nucleotide sequence encoding a complementary determining region (CDR). In one embodiment, the substitution of at least one non-histidine codon with a histidine codon is in the nucleotide sequence encoding a CDR3. In one embodiment, the substitution is of one, two, three, four, or more CDR3 codons. In one aspect, the single

rearranged human immunoglobulin light chain variable region sequence comprised at the immunoglobulin light chain locus is derived from a human Vκ1-39 or Vκ3-20 gene segment. In one embodiment, the single rearranged human immunoglobulin light chain variable region is derived from a rearranged Vκ1-39/Jκ5 or Vκ3-20/Jκ1 gene sequence. In one embodiment, the single rearranged human immunoglobulin light chain variable region is derived from a rearranged Vκ1-39/Jκ5 gene sequence, and the Vκ1-39/Jκ5 gene sequence comprises a replacement of at least one non-histidine codon with a histidine codon designed to express a histidine at a position selected from 105, 106, 108, 111, and a combination thereof. In another embodiment, the single rearranged human immunoglobulin light chain variable region is derived from a rearranged Vκ3-20/Jκ1 gene sequence, and the Vκ3-20/Jκ1 gene sequence comprises a replacement of at least one non-histidine codon with a histidine codon designed to express a histidine at a position selected from 105, 106, 107, 109, and a combination thereof.

[0014] In one aspect, the non-human animal described herein comprises a population of B cells in response to an antigen of interest that is enriched for antibodies that exhibit a decrease in dissociative half-life ($t_{1/2}$) at an acidic pH as compared to neutral pH of 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. In one embodiment, the decrease in $t_{1/2}$ at an acidic pH as compared to a neutral pH is about 30 fold or more.

[0015] In one embodiment, the animal expresses an antibody comprising a human immunoglobulin light chain variable domain with a substitution of at least one non-histidine residue with a histidine residue at an amino acid position encoded by the at least one codon substituted in the immunoglobulin light chain variable region gene sequence. In one embodiment, the animal expresses an antibody that retains a substitution of at least one non-histidine residue with a histidine residue in an expressed human immunoglobulin light chain variable domain, despite somatic hypermutations.

[0016] In one embodiment, the non-human animal is a mammal. In one embodiment, the mammal is a rodent, e.g., a rat or a mouse. In one embodiment, the non-human animal is a mouse. Thus, in one aspect, also provided herein is a genetically modified mouse comprising in its germline an immunoglobulin light chain locus that comprises a single rearranged human immunoglobulin light chain variable region gene sequence comprising human V_L and J_L segment sequences, wherein the single rearranged human immunoglobulin light chain variable region sequence comprises a substitution of at least one

non-histidine codon with a histidine codon. In one embodiment, the mouse lacks a functional unrearranged immunoglobulin light chain variable region.

[0017] In one embodiment, the single rearranged immunoglobulin light chain variable region gene sequence in the germline of the mouse is operably linked to an immunoglobulin light chain constant region gene sequence. In one embodiment, the immunoglobulin light chain constant region gene sequence is selected from a rat or a mouse immunoglobulin light chain constant region gene sequence. In one embodiment, the immunoglobulin light chain constant region gene sequence is a mouse sequence. In one embodiment, the immunoglobulin light chain locus is at an endogenous mouse immunoglobulin light chain locus.

[0018] In a further embodiment, the mouse also comprises in its germline an immunoglobulin heavy chain locus that comprises an unrearranged immunoglobulin heavy chain variable region sequence comprising human V_H, D_H, and J_H segments operably linked to an immunoglobulin heavy chain constant region gene sequence. In one aspect, the immunoglobulin heavy chain constant region gene sequence is a rat or a mouse heavy chain constant region gene sequence. In one embodiment, the immunoglobulin heavy chain constant region gene sequence is a mouse sequence. In one embodiment, the immunoglobulin heavy chain locus is at an endogenous mouse immunoglobulin heavy chain locus.

[0019] In one aspect, the mouse comprises a substitution of at least one non-histidine codon with a histidine codon wherein the substitution is in the nucleotide sequence encoding a CDR. In one embodiment, the substitution is in a CDR3 codon, e.g., in one, two, three, four, or more CDR3 codons. In one embodiment, the immunoglobulin light chain locus of the mouse comprises the single rearranged human immunoglobulin light chain variable region sequence derived from a human Vκ1-39 or Vκ3-20 gene segment, e.g., the single rearranged immunoglobulin light chain variable region sequence is derived from a rearranged Vκ1-39/Jκ5 or Vκ3-20/Jκ1 gene sequence. In one embodiment, the single rearranged immunoglobulin light chain variable region sequence is derived from a rearranged Vκ1-39/Jκ5 gene sequence and the Vκ1-39/Jκ5 sequence comprises a replacement of at least one non-histidine codon with a histidine codon designed to express a histidine at a position selected from 105, 106, 108, 111, and a combination thereof. In one embodiment, such replacement is designed to replace histidines at positions 105, 106, 108, and 111. In another embodiment, such replacement is designed to replace histidines at positions 106, 108, and 111.

[0020] In another embodiment, the single rearranged immunoglobulin light chain variable region sequence is derived from a rearranged Vκ3-20/Jκ1 gene sequence and the Vκ3-20/Jκ1 sequence comprises a replacement of at least one non-histidine codon with a histidine codon designed to express a histidine at a position selected from 105, 106, 107, 109, and a combination thereof. In one embodiment, such replacement is designed to replace histidines at positions 105, 106, 107, and 109. In another embodiment, such replacement is designed to replace histidines at positions 105, 106, and 109.

[0021] In one embodiment, the mouse described herein comprises a population of B cells in response to an antigen of interest that is enriched for antibodies that exhibit a decrease in dissociative half-life ($t_{1/2}$) at an acidic pH as compared to neutral pH of 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. In one embodiment, the decrease in $t_{1/2}$ at an acidic pH as compared to a neutral pH is about 30 fold or more.

[0022] In one embodiment, the mouse described herein expresses a population of antigen-specific antibodies in response to an antigen of interest wherein all antibodies comprise (a) immunoglobulin light chain variable domains derived from the same single rearranged human light chain variable region gene sequence which comprises a substitution of at least one non-histidine codon with a histidine codon, and (b) immunoglobulin heavy chains comprising heavy chain variable domains derived from a repertoire of human heavy chain V, D, and J segments.

[0023] Also provided herein is a non-human locus, e.g., mouse locus, comprising a single rearranged human immunoglobulin light chain variable region gene sequence comprising human V_L and J_L segment sequences, wherein the single rearranged human immunoglobulin light chain variable region gene sequence comprises a substitution of at least one non-histidine codon with a histidine codon. In one embodiment, the locus is comprised in the germline of a non-human animal. In one embodiment, the locus comprises the single rearranged human immunoglobulin light chain variable region gene sequence derived from a human Vκ1-39 or Vκ3-20 gene segment, e.g., derived from a rearranged Vκ1-39/Jκ5 or Vκ3-20/Jκ1 gene sequence. In one embodiment, wherein the single rearranged human immunoglobulin light chain variable region gene sequence present in the locus is derived from the rearranged Vκ1-39/Jκ5 sequence, the substitution of at least one non-histidine codon with a histidine codon is designed to express a histidine at a position selected from 105, 106, 108, 111, and a combination thereof. In another embodiment, wherein the single rearranged human immunoglobulin light chain variable region gene

sequence present in the locus is derived from the rearranged Vκ3-20/Jκ1 sequence, the substitution of at least one non-histidine codon with a histidine codon is designed to express a histidine at a position selected from 105, 106, 107, 109, and a combination thereof. In various embodiments, the non-human loci described herein may be generated using methods described below for making a genetically modified non-human animal.

[0024] In yet another aspect, provided herein is a method for making a non-human animal that comprises a genetically modified immunoglobulin light chain locus in its germline, wherein the method comprises modifying a genome of a non-human animal to delete or render non-functional endogenous immunoglobulin light chain V and J segments in an immunoglobulin light chain locus, and placing in the genome a single rearranged human light chain variable region gene sequence comprising a substitution of at least one non-histidine codon with a histidine codon. In one embodiment, such method results in a genetically modified non-human animal that comprises a population of B cells enriched for antibodies exhibiting pH-dependent binding to the antigen of interest. In one embodiment, the single rearranged human immunoglobulin light chain variable region sequence placed in the genome is derived from a human Vκ1-39 or Vκ3-20, e.g., a rearranged Vκ1-39/Jκ5 or Vκ3-20/Jκ1 gene sequence. Thus, in the embodiment wherein the single rearranged human immunoglobulin light chain variable region sequence is derived from a rearranged Vκ1-39/Jκ5, the substitution of at least one non-histidine codon with a histidine codon is designed to express a histidine at a position selected from 105, 106, 108, 111, and a combination thereof. In an embodiment wherein the single rearranged human immunoglobulin light chain variable region sequence is derived from a rearranged Vκ3-20/Jκ1, the substitution of at least one non-histidine codon with a histidine codon is designed to express a histidine at a position selected from 105, 106, 107, 109, and a combination thereof.

[0025] In another aspect, provided herein is a method of generating an antibody that exhibits pH-dependent binding to an antigen of interest comprising (a) generating a mouse described herein (e.g., a mouse that comprises in its germline an immunoglobulin light chain locus that comprises a single rearranged human immunoglobulin light chain variable region sequence comprising human V_L and J_L segment sequences and a substitution of at least one non-histidine codon with a histidine codon in its rearranged light chain variable region sequence), (b) immunizing the mouse with an antigen of interest, and (c) selecting an antibody that binds to the antigen of interest with a desired affinity at a neutral pH while displaying reduced binding to the antigen at an acidic pH. In one embodiment, the method results in a generation of an antibody that exhibits $t_{1/2}$ at acidic pH and 37°C of about 2 minutes or less. In one embodiment, the method results in a generation of an antibody that displays a decrease in dissociative half-life ($t_{1/2}$) at an acidic pH as compared to neutral pH

of 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.

[0026] In other aspects, provided herein are additional methods of generating an antibody that exhibits pH-dependent binding to an antigen of interest. One such method comprises (a) selecting a first antibody that binds to an antigen of interest with a desired affinity, (b) modifying an immunoglobulin light chain nucleotide sequence of the first antibody to comprise a substitution of at least one non-histidine codon with a histidine codon, (c) expressing an immunoglobulin heavy chain of the first antibody and the modified immunoglobulin light chain in a cell, and (d) selecting a second antibody expressed in the cell that retains a desired affinity for the antigen of interest at neutral pH and displays reduced binding to the antigen of interest at an acidic pH. In one embodiment, the immunoglobulin light chain nucleotide sequence of the first antibody comprises a single rearranged human immunoglobulin light chain variable region sequence. In one embodiment, the first antibody is generated in a non-human animal, e.g., a mouse, comprising an immunoglobulin light chain sequence derived from a single rearranged human immunoglobulin light chain variable region sequence, and the modification of the immunoglobulin light chain is made in the single rearranged human immunoglobulin variable region sequence. In one embodiment, the first antibody is generated in a non-human animal, e.g., a mouse, further comprising an immunoglobulin heavy chain sequence derived from a repertoire of human V_H, D_H, and J_H segments. In one embodiment, the single rearranged human immunoglobulin light chain variable region sequence is selected from Vκ1-39/Jκ5 and Vκ3-20/Jκ1 gene sequence. In an embodiment, wherein the single rearranged human immunoglobulin light chain variable region sequence is Vκ1-39/Jκ5, the modification in the immunoglobulin light chain nucleotide sequence of the first antibody is made in the CDR3 codon at a position selected from 105, 106, 108, 111, and a combination thereof. In an embodiment wherein the single rearranged human immunoglobulin light chain variable region sequence is Vκ3-20/Jκ1, the modification in the immunoglobulin light chain nucleotide sequence of the first antibody is made in the CDR3 codon at a position selected from 105, 106, 107, 109, and a combination thereof.

[0027] In one embodiment, the method of generating an antibody that exhibits pH-dependent binding to an antigen of interest described herein results in an antibody that displays a decrease in dissociative half-life ($t_{1/2}$) at an acidic pH as compared to neutral pH of 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. In one embodiment, the method of generating the antibody results in an antibody that exhibits a $t_{1/2}$ at acidic pH and 37°C of about 2 minutes or less.

[0028] Any of the embodiments and aspects described herein can be used in conjunction with one another, unless otherwise indicated or apparent from the context. Other embodiments will become apparent to those skilled in the art from a review of the ensuing description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] FIG. 1 illustrates an amino acid alignment of human Vκ1-39-derived light chains from various antigen-specific antibodies (A-K antibodies). Histidine (H) residues located within each light chain sequence are in bold. Various light chain regions (Framework and CDR) are indicated above the alignment.

[0030] FIG. 2 illustrates the combinations and locations of histidine residues engineered in the CDR3 region of human Vκ1-39-derived light chains by mutagenesis. Corresponding nucleic acid sequences are included. Histidine residues introduced through mutagenesis and corresponding nucleic acid residues are shown in bold. Amino acid positions (105, 106, etc.) are based on a unique numbering described in Lefranc et al. (2003) Dev. Comp. Immunol. 27:55-77, and can also be viewed on www.imgt.org.

[0031] FIG. 3 illustrates the level of antibody expression in ng/mL detected in the supernatants of CHO cells transfected with nucleic acids encoding five (1-5) different heavy chains and Vκ1-39-derived light chains having histidine residues engineered at indicated locations (see Y axis) in the CDR3.

[0032] FIG. 4 is a western blot showing expression of selected antigen-specific human antibodies containing histidine engineered light chains in CHO cell supernatants.

[0033] FIGs. 5A-5E show the binding kinetics for selected heavy chains (1-5) from antigen-specific antibodies paired with various histidine engineered light chains at a neutral (7.4) and acidic (5.75) pH. Various kinetic parameters including k_a, k_d, K_D, and $t_{1/2}$ are shown. NB=no binding.

[0034] FIG. 6 shows kinetic parameters (K_D and $t_{1/2}$) for antibodies comprising parental universal light chain or histidine-modified universal light chain paired with indicated heavy chains (2, 3, and 6). Histidine substitutions lead to strong pH dependence in several antibodies. Histidine substitutions were made in CDR3 to convert the sequence $_{105}$QQSYSTP$_{111}$ (SEQ ID NO:3) to $_{105}$HHSYSTH$_{111}$ (SEQ ID NO:5). Note that NB = no binding detected (KD > 10 micromolar).

[0035] FIG. 7 shows the sequence and properties (%GC content, N, % mismatch, Tm) of selected mutagenesis primers used to engineer histidine residues into CDR3 of a rearranged human Vκ1-39/Jκ5 light chain sequence. SEQ ID NOs for these primers used in the Sequence Listing are included in the Table below. F=forward primer, R=reverse primer.

[0036] FIGs. 8A – 8B show a general strategy for construction of targeting vectors for engineering of histidine residues into a rearranged human light chain variable region sequence derived from Vκ1-39/Jκ5 variable region for making a genetically modified mouse that expresses antibodies containing the modified human light chain. FIGs. 8C-8D show introduction of the targeting vector for ULC-H105/106/108/111 substitutions into ES cells and generation of heterozygous mice from the same; while FIGs. 8E-8F show introduction of the targeting vector for ULC-H106/108/111 substitutions into ES cells and generation of heterozygous mice from the same. The diagrams are not presented to scale. Unless indicated otherwise, filled shapes and solid lines represent mouse sequence, empty shapes and double lines represent human sequence.

[0037] FIG. 9 shows antiserum titers against immunogen from mice heterozygous for histidine universal light chain (HULC) (with 4 His substitutions – HULC 1927 mice; with 3 His substitutions – HULC 1930 mice) and wild type animals in a second bleed.

[0038] FIG. 10 is a comparison of the number of total antigen positive clones and the number of antigen positive clones displaying pH sensitive antigen binding obtained from hybridoma fusions from heterozygous HULC (1927 vs 1930) and WT mice. Figure includes data for two mice for each mouse type ("mouse 1" and "mouse 2").

[0039] FIGs. 11A-11C show sensorgrams from surface plasmon resonance binding experiments in which monoclonal antibodies (AA, BB, CC, DD, HH, GG, NN, and OO) from either heterozygous HULC or WT mice were allowed to associate with the immunogen at neutral pH (pH 7.4) followed by a shift to a buffer with pH of either 7.4 or 6.0 for the dissociation phase. The individual lines in each graph represent the binding responses at different concentrations of the respective antibodies. All experiments were carried out at 25ºC. Dissociative half-life values (t½) are noted above the respective sensorgrams, and fold change in t½ is included to the right of each sensorgram. Antibodies AA, BB, CC, DD, HH, and GG were from heterozygous HULC 1927 mice using His-substituted light chain, NN is from heterozygous HULC 1927 mouse using WT light chain, and OO is from a WT mouse (See Table 4 for clarification).

[0040] FIG. 12 shows positions of histidine residues engineered in the CDR3 region of human Vκ3-20-derived light chains by mutagenesis. Histidine residues introduced through mutagenesis and corresponding nucleic acid residues are shown in bold. Amino acid

positions (105, 106, etc.) are based on a unique numbering described in Lefranc et al. (2003) Dev. Comp. Immunol. 27:55-77, and can also be viewed on www.imgt.org.

[0041] FIG. 13 shows the sequence and properties (%GC content, N, % mismatch, Tm) of selected mutagenesis primers used to engineer histidine residues into CDR3 of a rearranged human Vκ3-20/Jκ1 light chain sequence. SEQ ID NOs for these primers used in the Sequence Listing are included in the Table below. F=forward primer, R=reverse primer.

[0042] FIGs. 14A – 14B show a general strategy for construction of targeting vectors for the engineering of histidine residues into a rearranged human light chain variable region sequence derived from Vκ3-20/Jκ1 light chain variable region for making a genetically modified mouse that expresses antibodies containing the modified human light chain. FIG 14C shows introduction of the targeting vector for ULC-Q105H/Q106H/Y107H/S109H substitutions into ES cells and generation of heterozygous mice from the same; while FIG. 14D shows introduction of the targeting vector for ULC-Q105H/Q106H/S109H substitutions into ES cells and generation of heterozygous mice from the same. The diagrams are not presented to scale. Unless indicated otherwise, filled shapes and solid lines represent mouse sequence, empty shapes and double lines represent human sequence.

DETAILED DESCRIPTION OF INVENTION

Definitions

[0043] The present invention provides genetically modified non-human animals (e.g., mice, rats, rabbits, hamsters, etc.) that comprise in their genome, e.g., in their germline, nucleotide sequence(s) encoding human antibody molecules that exhibit pH-dependent antigen binding, e.g., a nucleotide sequence of immunoglobulin light chain comprising rearranged human immunoglobulin light chain variable region sequence encoding antibodies that exhibits pH-dependent antigen binding; embryos, cells, and tissues comprising the same; methods of making the same; as well as methods of using the same. 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.

[0044] The term "antibody", as used herein, includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain comprises a heavy chain variable domain and a heavy chain constant region (C_H). The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3. Each light chain comprises a light chain variable domain and a light chain constant region (C_L). The heavy chain and light chain variable domains can be further subdivided into regions of hypervariability, termed complementarity determining

regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each heavy and light chain variable domain comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 (heavy chain CDRs may be abbreviated as HCDR1, HCDR2 and HCDR3; light chain CDRs may be abbreviated as LCDR1, LCDR2 and LCDR3). The term "high affinity" antibody refers to an antibody that has a K_D with respect to its target epitope about of 10^{-9} M or lower (e.g., about 1 x 10^{-9} M, 1 x 10^{-10} M, 1 x 10^{-11} M, or about 1 x 10^{-12} M). In one embodiment, K_D is measured by surface plasmon resonance, e.g., BIACORE™; in another embodiment, K_D is measured by ELISA.

[0045] The phrase "bispecific antibody" includes an antibody capable of selectively binding two or more epitopes. Bispecific antibodies generally comprise two nonidentical heavy chains, with each heavy chain specifically binding a different epitope—either on two different molecules (e.g., different epitopes on two different immunogens) or on the same molecule (e.g., different epitopes on the same immunogen). If a bispecific antibody is capable of selectively binding two different epitopes (a first epitope and a second epitope), the affinity of the first heavy chain for the first epitope will generally be at least one to two or three or four or more orders of magnitude lower than the affinity of the first heavy chain for the second epitope, and vice versa. Epitopes specifically bound by the bispecific antibody can be on the same or a different target (e.g., on the same or a different protein). Exemplary bispecific antibodies include those with a first heavy chain specific for a tumor antigen and a second heavy chain specific for a cytotoxic marker, *e.g.,* an Fc receptor (*e.g.,* FcγRI, FcγRII, FcγRIII, etc.) or a T cell marker (*e.g.,* CD3, CD28, etc.). Further, the second heavy chain variable domain can be substituted with a heavy chain variable domain having a different desired specificity. For example, a bispecific antibody with a first heavy chain specific for a tumor antigen and a second heavy chain specific for a toxin can be paired so as to deliver a toxin (*e.g.,* saporin, vinca alkaloid, *etc.*) to a tumor cell. Other exemplary bispecific antibodies include those with a first heavy chain specific for an activating receptor (*e.g.,* B cell receptor, FcγRI, FcγRIIA, FcγRIIIA, FcαRI, T cell receptor, *etc.*) and a second heavy chain specific for an inhibitory receptor (*e.g.,* FcγRIIB, CD5, CD22, CD72, CD300a, *etc.*). Such bispecific antibodies can be constructed for therapeutic conditions associated with cell activation (*e.g.* allergy and asthma). Bispecific antibodies can be made, for example, by combining heavy chains that recognize different epitopes of the same immunogen. For example, nucleic acid sequences encoding heavy chain variable sequences that recognize different epitopes of the same immunogen can be fused to nucleic acid sequences encoding the same or different heavy chain constant regions, and such sequences can be expressed in a cell that expresses an immunoglobulin light chain. A typical bispecific antibody has two

heavy chains each having three heavy chain CDRs, followed by (N-terminal to C-terminal) a C_H1 domain, a hinge, a C_H2 domain, and a C_H3 domain, and an immunoglobulin light chain that either does not confer epitope-binding specificity but that can associate with each heavy chain, or that can associate with each heavy chain and that can bind one or more of the epitopes bound by the heavy chain epitope-binding regions, or that can associate with each heavy chain and enable binding of one or both of the heavy chains to one or both epitopes.

[0046] The term “cell” includes any cell that is suitable for expressing a recombinant nucleic acid sequence. Cells include those of prokaryotes and eukaryotes (single-cell or multiple-cell), bacterial cells (e.g., strains of E. coli, Bacillus spp., Streptomyces spp., etc.), mycobacteria cells, fungal cells, yeast cells (e.g., *S. cerevisiae, S. pombe, P. pastoris, P. methanolica*, etc.), plant cells, insect cells (e.g., SF-9, SF-21, baculovirus-infected insect cells, Trichoplusia ni, etc.), non-human animal cells, human cells, or cell fusions such as, for example, hybridomas or quadromas. In some embodiments, the cell is a human, monkey, ape, hamster, rat, or mouse cell. In some embodiments, the cell is eukaryotic and is selected from the following cells: CHO (e.g., CHO K1, DXB-11 CHO, Veggie-CHO), COS (e.g., COS-7), retinal cell, Vero, CV1, kidney (e.g., HEK293, 293 EBNA, MSR 293, MDCK, HaK, BHK), HeLa, HepG2, WI38, MRC 5, Colo205, HB 8065, HL-60, (e.g., BHK21), Jurkat, Daudi, A431 (epidermal), CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT 060562, Sertoli cell, BRL 3A cell, HT1080 cell, myeloma cell, tumor cell, and a cell line derived from an aforementioned cell. In some embodiments, the cell comprises one or more viral genes, e.g. a retinal cell that expresses a viral gene (e.g., a PER.C6™ cell).

[0047] The phrase “complementarity determining region,” or the term “CDR,” 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 or unrearranged sequence, and, for example, by a naive 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 the result of splicing or connecting the sequences (e.g., V-D-J recombination to form a heavy chain CDR3).

[0048] The term "conservative,” when used to describe a conservative amino acid substitution, includes substitution of an amino acid residue by another amino acid residue

having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of interest of a protein, for example, the ability of a variable region to specifically bind a target epitope with a desired affinity. Examples of groups of amino acids that have side chains with similar chemical properties include aliphatic side chains such as glycine, alanine, valine, leucine, and isoleucine; aliphatic-hydroxyl side chains such as serine and threonine; amide-containing side chains such as asparagine and glutamine; aromatic side chains such as phenylalanine, tyrosine, and tryptophan; basic side chains such as lysine, arginine, and histidine; acidic side chains such as aspartic acid and glutamic acid; and, sulfur-containing side chains such as cysteine and methionine. Conservative amino acids substitution groups include, for example, valine/leucine/isoleucine, phenylalanine/tyrosine, lysine/arginine, alanine/valine, glutamate/aspartate, and asparagine/glutamine. In some embodiments, a conservative amino acid substitution can be substitution of any native residue in a protein with alanine, as used in, for example, alanine scanning mutagenesis. In some embodiments, a conservative substitution is made that has a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al. (1992) Exhaustive Matching of the Entire Protein Sequence Database, Science 256:1443-45, hereby incorporated by reference. In some embodiments, the substitution is a moderately conservative substitution wherein the substitution has a nonnegative value in the PAM250 log-likelihood matrix.

[0049] In some embodiments, residue positions in an immunoglobulin light chain or heavy chain differ by one or more conservative amino acid substitutions. In some embodiments, residue positions in an immunoglobulin light chain or functional fragment thereof (e.g., a fragment that allows expression and secretion from, e.g., a B cell) are not identical to a light chain whose amino acid sequence is listed herein, but differs by one or more conservative amino acid substitutions.

[0050] The phrase “epitope-binding protein” includes a protein having at least one CDR and that is capable of selectively recognizing an epitope, e.g., is capable of binding an epitope with a K_D that is at about one micromolar or lower (e.g., a K_D that is about 1×10^{-6} M, 1×10^{-7} M, 1×10^{-9} M, 1×10^{-9} M, 1×10^{-10} M, 1×10^{-11} M, or about 1×10^{-12} M). Therapeutic epitope-binding proteins (e.g., therapeutic antibodies) frequently require a K_D that is in the nanomolar or the picomolar range.

[0051] The phrase “functional fragment” includes fragments of epitope-binding proteins that can be expressed, secreted, and specifically bind to an epitope with a K_D in the micromolar, nanomolar, or picomolar range. Specific recognition includes having a K_D that is at least in the micromolar range, the nanomolar range, or the picomolar range.

[0052] The term “germline” in reference to an immunoglobulin nucleic acid sequence includes a nucleic acid sequence that can be passed to progeny.

[0053] 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. A heavy chain variable domain is encoded by a variable region gene 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, www.imgt.org.

[0054] The term “identity” when used in connection with sequence, includes identity as determined by a number of different algorithms known in the art that can be used to measure nucleotide and/or amino acid sequence identity. In some embodiments described herein, identities are determined using a ClustalW v. 1.83 (slow) alignment employing an open gap penalty of 10.0, an extend gap penalty of 0.1, and using a Gonnet similarity matrix (MACVECTOR™ 10.0.2, MacVector Inc., 2008). The length of the sequences compared with respect to identity of sequences will depend upon the particular sequences, but in the case of a light chain constant domain, the length should contain sequence of sufficient length to fold into a light chain constant domain that is capable of self-association to form a canonical light chain constant domain, e.g., capable of forming two beta sheets comprising beta strands and capable of interacting with at least one C_H1 domain of a human or a mouse. In the case of a C_H1 domain, the length of sequence should contain sequence of sufficient length to fold into a C_H1 domain that is capable of forming two beta sheets comprising beta strands and capable of interacting with at least one light chain constant domain of a mouse or a human.

[0055] The phrase “immunoglobulin molecule” includes two immunoglobulin heavy chains and two immunoglobulin light chains. The heavy chains may be identical or different, and the light chains may be identical or different.

[0056] The phrase “light chain” includes an immunoglobulin light chain sequence from any organism, and unless otherwise specified includes human kappa and lambda 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. A light chain variable domain is encoded by a light chain variable region gene sequence, which generally comprises V_L and J_L segments, derived from a repertoire of V and J segments present in the germline. Sequences, locations and nomenclature for V and J light chain segments for various organisms can be found in IMGT database, www.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. Common or universal light chains include those derived from a human Vκ1-39Jκ5 gene or a human Vκ3-20Jκ1 gene, and include somatically mutated (e.g., affinity matured) versions of the same.

[0057] The phrase “micromolar range” is intended to mean 1-999 micromolar; the phrase “nanomolar range” is intended to mean 1-999 nanomolar; the phrase “picomolar range” is intended to mean 1-999 picomolar.

[0058] The phrase “somatically mutated” 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., nucleotide sequence encoding 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 framework nucleic acid sequence between a B cell that has not undergone class-switching and a B cell that has undergone class-switching. “Somatically mutated” includes reference to nucleic acid sequences from affinity-matured B cells that are not identical to corresponding immunoglobulin variable region sequences in B cells that are not affinity-matured (i.e., sequences in the genome of germline cells). The phrase “somatically mutated” 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 phrase “somatically mutated” 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.

[0059] The term “unrearranged,” with reference to a nucleic acid sequence, includes nucleic acid sequences that exist in the germline of an animal cell.

[0060] The phrase “variable domain” includes an amino acid sequence of an immunoglobulin light or heavy chain (modified as desired) that comprises the following amino acid regions, in sequence from N-terminal to C-terminal (unless otherwise indicated): FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[0061] The term "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.

[0062] The term “replacement” in reference to gene replacement refers to placing exogenous genetic material at an endogenous genetic locus, thereby replacing all or a portion of the endogenous gene with an orthologous or homologous nucleic acid sequence.

[0063] “Functional” as used herein, e.g., in reference to a functional polypeptide, includes a polypeptide that retains at least one biological activity normally associated with the native protein. In another instance, a functional immunoglobulin gene segment may include a variable gene segment that is capable of productive rearrangement to generate a rearranged immunoglobulin gene sequence.

[0064] “Neutral pH” includes pH between about 7.0 and about 8.0, e.g., pH between about 7.0 and about 7.4, e.g., between about 7.2 and about 7.4, e.g., physiological pH. “Acidic pH” includes pH of 6.0 or lower, e.g., pH between about 5.0 and about 6.0, pH between about 5.75 and about 6.0, e.g., pH of endosomal or lysosomal compartments.

Engineered Histidine Residues In Immunoglobulin Light Chain Genes

[0065] The inventors have discovered that non-human animals that express antibodies that are capable of binding to an antigen in a pH dependent manner can be made by making modifications of an immunoglobulin light chain variable region at one or more positions along the sequence of the light chain. Methods of making modifications in the germline of a non-human animal so that the animal would express histidines in CDRs of antibodies are

described. In particular, methods for making modifications in an immunoglobulin light chain variable sequence in the germline of the mouse are described. Variable region sequence, *e.g.,* of light chains, typically show somatic hypermutation along the variable region sequence, and, in some cases, such mutations can result in a substitution of histidine residues (see, e.g., FIG. 1). Such mutations can even occur in complementary determining regions (CDRs), which are the regions of variable domains responsible for antigen binding. In some cases, such mutations can result in antibodies that display pH-dependent antigen binding, e.g., reduced antigen binding at an acidic pH as compared to antigen binding at a neutral pH. Such pH-dependent antigen binding is desired because it may enable the antibody to bind to the antigen outside the cell, and, when internalized into an endosome, release the antigen and recycle back to the surface to bind another antigen, avoiding target-mediated clearance. Approaches for introducing histidine residues to achieve this effect by using a *random* his-scanning mutagenesis to engineer pH-dependent binding properties in anti-IL-6R antibodies have been reported (US 2011/0111406 A1). However, random mutagenesis of antibody residues may result in decreased affinity of antibody to the antigen. A non-human animal genetically modified to express a histidine substitution in antibody sequence enables generation of high-affinity antibodies in response to an antigen of interest that, due to histidine modification(s), would also display pH-dependent antigen binding.

[0066] Thus, in various embodiments, provided herein is a genetically modified non-human animal (e.g., rodent, e.g., a mouse or a rat) that comprises in its genome, e.g., in its germline, a human immunoglobulin light chain variable region sequence comprising modifications that result in the animal expressing antibodies capable of binding to antigens in a pH-dependent manner. In one embodiment, the non-human animal comprises modifications in the human immunoglobulin light chain variable region sequence (e.g., V_L and/or J_L segment sequence) that comprise substitutions in at least one non-histidine codon with a histidine codon (in some cases, also may be referred to as “histidine substitution,” “histidine codon substitution,” or the like). In one embodiment, the animal comprises at least one substitution of a non-histidine codon with a histidine codon in a nucleotide sequence of a complementary determining region (CDR; e.g., CDR1, CDR2, and/or CDR3) of a human immunoglobulin light chain. In one embodiment, the substitution is in a CDR3 codon. In one embodiment, the light chain is a κ light chain. In one embodiment, the animal expresses an immunoglobulin light chain, e.g., a light chain CDR, e.g., a light chain CDR3, comprising a substitution of at least one amino acid with a histidine. In another embodiment, the light chain is a λ light chain. In yet another embodiment, the mouse comprises a substitution of at least one non-histidine codon with a histidine codon in both κ and λ light chains.

[0067] Histidine residue is encoded by two different codons, CAT and CAC (deoxyribonucleic acid residues). Thus, a non-histidine codon may be substituted with a CAT or a CAC. The substitution is engineered in a codon that in its germline configuration (i.e., non-somatically mutated state) does not encode a histidine residue.

[0068] In one embodiment a light chain is a universal light chain (also termed a common light chain). As described in U.S. Patent Application Nos. 13/022,759, 13/093,156, 13/412,936 and 13/488,628 (U.S. Application Publication Nos. 2011/0195454, 2012/0021409, 2012/0192300 and 2013/0045492, all incorporated herein by reference), a non-human animal (e.g., a mouse) that selects a common light chain for a plurality of heavy chains has a practical utility. In various embodiments, antibodies expressed in a non-human animal comprising only a common light chain will have heavy chains that can associate and express with an identical or substantially identical light chain. This is particularly useful in making bispecific antibodies. For example, such an animal can be immunized with a first immunogen to generate a B cell that expresses an antibody that specifically binds a first epitope. The animal (or an animal genetically the same) can be immunized with a second immunogen to generate a B cell that expresses an antibody that specifically binds the second epitope. Variable heavy chain regions can be cloned from the B cells and expressed with the same heavy chain constant region and the same light chain (e.g., a common light chain) in a cell to make a bispecific antibody, wherein the heavy chain component of the bispecific antibody has been selected by an animal to associate and express with the same light chain component. In various embodiments described, the variable regions of the genetically engineered mice are human variable regions.

[0069] Thus, a mouse was engineered that is capable of generating immunoglobulin light chains that will suitably pair with a rather diverse family of heavy chains, including heavy chains whose human variable regions depart from germline sequences, *e.g.,* affinity matured or somatically mutated variable regions. In various embodiments, the mouse is devised to pair human light chain variable domains with human heavy chain variable domains that comprise somatic mutations, thus enabling a route to high affinity binding proteins suitable for use as human therapeutics.

[0070] The genetically engineered mouse, through the long and complex process of antibody selection within an organism, makes biologically appropriate choices in pairing a diverse collection of human heavy chain variable domains with a limited number of human light chain options. In order to achieve this, the mouse is engineered to present a limited number of human light chain variable domain options in conjunction with a wide diversity of human heavy chain variable domain options. Upon challenge with an immunogen, the mouse maximizes the number of solutions in its repertoire to develop an antibody to the

immunogen, limited largely or solely by the number or light chain options in its repertoire. In various embodiments, this includes allowing the mouse to achieve suitable and compatible somatic mutations of the light chain variable domain that will nonetheless be compatible with a relatively large variety of human heavy chain variable domains, including in particular somatically mutated human heavy chain variable domains.

[0071] The engineered common light chain mouse described in U.S. Application Publication Nos. 2011/0195454, 2012/0021409, 2012/0192300 and 2013/0045492 comprised nucleic acid sequence encoding a limited repertoire of light chain options, e.g., common or universal light chain "ULC" that comprised no more than two V_L segments or a single rearranged human immunoglobulin light chain variable region sequence. To achieve such limited repertoire, a mouse was engineered to render nonfunctional or substantially nonfunctional its ability to make, or rearrange, a native mouse light chain variable domain. In one aspect, this was achieved, *e.g.,* by deleting the mouse's light chain variable region gene segments. As previously described, the endogenous mouse locus can then be modified by exogenous suitable human light chain variable region gene segments of choice, operably linked to the endogenous mouse light chain constant domain, in a manner such that the exogenous human variable region gene segments can combine with the endogenous mouse light chain constant region gene and form a rearranged reverse chimeric light chain gene (human variable, mouse constant). In various embodiments, the light chain variable region is capable of being somatically mutated. In various embodiments, to maximize ability of the light chain variable region to acquire somatic mutations, the appropriate enhancer(s) is retained in the mouse. In one aspect, in modifying a mouse κ light chain locus to replace endogenous mouse κ light chain gene segments with human κ light chain gene segments, the mouse κ intronic enhancer and mouse κ 3' enhancer are functionally maintained, or undisrupted.

[0072] Thus, provided was a genetically engineered mouse that expresses a limited repertoire of reverse chimeric (human variable, mouse constant) light chains associated with a diversity of reverse chimeric (human variable, mouse constant) heavy chains. In various embodiments, the endogenous mouse κ light chain gene segments are deleted and replaced with a single (or two) rearranged human light chain region, operably linked to the endogenous mouse Cκ gene. In embodiments for maximizing somatic hypermutation of the rearranged human light chain region, the mouse κ intronic enhancer and the mouse κ 3' enhancer are maintained. In various embodiments, the mouse also comprises a nonfunctional λ light chain locus, or a deletion thereof or a deletion that renders the locus unable to make a λ light chain.

[0073] The universal light chain mouse generated antibodies in response to various antigens that were capable of utilizing a diverse repertoire of heavy chain variable region sequences, comprising a diverse repertoire of V_H, D_H, and J_H segments. Antibodies generated in such genetically engineered ULC mouse are useful for designing bispecific therapeutic antibodies; however, as with any other antibody, each bispecific antibody may only bind to one target during its lifetime in the plasma; the antibody is internalized into an endosome and targeted for lysosomal degradation. Studies have shown that MHC-class-I-like Fcγ receptor FcRn is capable of rescuing immunoglobulins from lysosomal degradation by recycling it back to the cell surface from the sorting endosome. Simister and Mostov (1989) An Fc receptor structurally related to MHC class I antigens. Nature 337: 184-87. As explained above, to improve efficiency of antibody recycling, further modifications to antibody sequences, e.g., modifications that result in decreased antigen binding at acidic pH (e.g., pH of the endosome), while retaining antibody-antigen affinity and specificity at neutral pH (e.g., physiological pH) are beneficial. The non-human animals described herein, wherein histidine residues are substituted for non-histidine residues in the a universal light chain sequence are beneficial because they are capable of producing high-affinity antibodies based on universal light chain format that also display pH-dependent binding, e.g., display reduced binding to the antigen at acidic versus neutral pH.

[0074] Thus, in one embodiment, provided herein is a non-human animal (e.g., a rodent, e.g., a mouse or a rat) that comprises in its genome, e.g., in its germline, a limited repertoire of human light chain variable regions, or a single human light chain variable region, from a limited repertoire of human light chain variable gene segments, wherein the human light chain variable region(s) comprise at least one substitution of a non-histidine codon for a histidine codon. In some embodiments, provided non-human animals are genetically engineered to include a single unrearranged human light chain variable region gene segment (or two human light chain variable region gene segments) that rearranges to form a rearranged human light chain variable region gene (or two rearranged light chain variable region genes) that expresses a single light chain (or that express either or both of two light chains), wherein the light chain variable region gene(s) comprise a substitution of at least one non-histidine codon with a histidine codon. The rearranged human light chain variable domains encoded by these histidine-substituted light chain variable region gene(s) are capable of pairing with a plurality of affinity-matured human heavy chains selected by the animals, wherein the heavy chain variable regions specifically bind different epitopes. In various embodiments, the at least one substitution of a non-histidine residue with a histidine residue results in a rearranged human light chain that, when expressed with a cognate heavy chain, binds to its antigen in a pH-dependent manner.

[0075] Genetically engineered animals are provided that express a limited repertoire of human light chain variable domains, or a single human light chain variable domain, from a limited repertoire of human light chain variable region gene sequences, wherein the variable region gene sequences comprise at least one substitution of a non-histidine codon with a histidine codon. In some embodiments, provided animals are genetically engineered to include a single V/J human light chain sequence (or two V/J sequences) that comprises a substitution of at least one non-histidine codon with a histidine codon and expresses a variable region of a single light chain (or that express either or both of two variable regions). In one aspect, a light chain comprising the variable sequence is capable of pairing with a plurality of affinity-matured human heavy chains clonally selected by the animal, wherein the heavy chain variable regions specifically bind different epitopes. In one embodiment, the antibody binds to its antigen(s) in a pH-dependent manner. In one embodiment, the single V/J human light chain sequence is selected from Vκ1-39Jκ5 and Vκ3-20Jκ1. In one embodiment, the two V/J sequences are Vκ1-39Jκ5 and Vκ3-20Jκ1. In one embodiment, the Vκ1-39Jκ5 and Vκ3-20Jκ1 sequences are rearranged V/J sequences.

[0076] In one aspect, a genetically modified non-human animal is provided that comprises a single human immunoglobulin light chain V_L gene segment that is capable of rearranging with a human J_L gene segment (selected from one or a plurality of J_L segments) and encoding a human variable domain of an immunoglobulin light chain, wherein the single human immunoglobulin light chain V_L gene segment and/or human J_L gene segment comprise a substitution of at least one non-histidine codon with a histidine codon. In another aspect, a genetically modified mouse is provided that comprises no more than two human V_L gene segments, each of which is capable of rearranging with a human J_L gene segment (selected from one or a plurality of J_L segments) and encoding a human variable domain of an immunoglobulin light chain, wherein each of the no more than two V_L gene segments and/or the J_L gene segment comprise a substitution of at least one non-histidine residue with a histidine residue.

[0077] Also provided herein is a genetically modified non-human animal that comprises in its genome, e.g., in its germline, a single rearranged human immunoglobulin light chain variable region sequence comprising human V_L and J_L sequences wherein the single rearranged human immunoglobulin light chain variable region comprises a substitution of at least one non-histidine codon with a histidine codon. In one aspect, the single rearranged human immunoglobulin light chain variable region sequence is derived from human germline V_L and J_L gene sequences, but for the histidine substitution(s). In one embodiment, the human immunoglobulin light chain is a human immunoglobulin κ chain. Thus, in one embodiment, the human V_L gene sequence is selected from Vκ1-39 and Vκ3-20. In one

embodiment, the single rearranged human immunoglobulin light chain variable region sequence comprises rearranged Vκ1-39/J or Vκ3-20/J sequence. In one embodiment, the human J_L gene sequence is selected from Jκ1, Jκ2, Jκ3, Jκ4, and Jκ5. In one embodiment the human J_L sequence is selected from Jκ1 and Jκ5. In one embodiment, the single rearranged human immunoglobulin light chain variable region sequence is selected from Vκ1-39Jκ5 and Vκ3-20Jκ1 (e.g., but for the histidine substitution(s)). In an alternative embodiment, the human immunoglobulin light chain is a human λ chain.

[0078] In one embodiment, the substitution of at least one non-histidine codon for a histidine codon is in the nucleotide sequence encoding a complementary determining region (CDR) of the light chain variable domain. In one embodiment, the substitution of at least one non-histidine codon for a histidine codon is in the nucleotide sequence encoding CDR1, CDR2 or CDR3 of the light chain variable domain. In one specific embodiment, the substitution is in the nucleotide sequence encoding CDR3.

[0079] In one aspect, the substitution is of at least one non-histidine codon for a histidine codon in the CDR3 codon of the human light chain variable region gene sequence. In one embodiment, the substitution is of one, two, three, four, or more CDR3 codons. In the embodiment wherein the single rearranged human immunoglobulin light chain variable region is a Vκ1-39Jκ5 variable region, the replacement of at least one non-histidine codon with a histidine codon comprises a replacement at a position in the immunoglobulin light chain gene sequence encoding CDR3 designed to express a histidine at position selected from 105, 106, 108, 111, and a combination thereof. In one embodiment, the replacement is designed to express histidines at positions 105 and 106. In one embodiment, the replacement is designed to express histidines at positions 105 and 111. In one embodiment, the replacement is designed to express histidines at positions 105 and 108. In one embodiment, the replacement is designed to express histidines at positions 105, 108 and 111. In one embodiment, the replacement is designed to express histidines at positions 105, 106, and 108. In one embodiment, the replacement is designed to express histidines at positions 106 and 108. In one embodiment, the replacement is designed to express histidines at positions 106 and 111. In one embodiment, the replacement is designed to express histidines at positions 108 and 111. In one embodiment, the replacement is designed to express histidines at positions 106, 108, and 111. In yet another embodiment, the replacement is designed to express histidines at positions 106, 108 and 111. In one embodiment, the replacement is designed to express histidines at positions 105, 106, and 111. In one embodiment, the replacement is designed to express histidines at positions 105, 106, 108, and 111. The nucleic acid and amino acid sequences of the histidine-substituted CDR3 regions are depicted in sequence alignment of FIG. 2 and set forth in SEQ

ID NOs: 4-33. Wild type CDR3 nucleic acid and amino acid sequences (depicted in FIG. 2) are set forth in SEQ ID NOs:2 and 3, respectively.

[0080] In the embodiment wherein the single rearranged human immunoglobulin light chain variable region is a Vκ3-20Jκ1 variable region, the replacement of at least one non-histidine codon with a histidine codon comprises a replacement at a position in the immunoglobulin light chain gene sequence encoding CDR3 region that is designed to express a histidine at position selected from 105, 106, 107, 109, and a combination thereof. In one embodiment, the replacement is designed to express histidines at positions 105 and 106. In one embodiment, the replacement is designed to express histidines at positions 105 and 107. In one embodiment, the replacement is designed to express histidines at positions 105 and 109. In one embodiment, the replacement is designed to express histidines at positions 106 and 107. In one embodiment, the replacement is designed to express histidines at positions 106 and 109. In one embodiment, the replacement is designed to express histidines at positions 107 and 109. In one embodiment, the replacement is designed to express histidines at positions 105, 106, and 107. In one embodiment, the replacement is designed to express histidines at positions 105, 107, and 109. In one embodiment, the replacement is designed to express histidines at positions 106, 108, and 111. In one embodiment, the replacement is designed to express histidines at positions 105, 106 and 109. In another embodiment, the replacement is designed to express histidines at positions 105, 106, 107, and 109. The nucleic acid and amino acid sequences of exemplary histidine-substituted CDR3 regions are depicted in sequence alignment of FIG. 12 and set forth in SEQ ID NOs: 76-79. Wild type CDR3 nucleic acid and amino acid sequences (depicted in FIG. 12) are set forth in SEQ ID NOs:74 and 75, respectively.

[0081] Amino acid positions (105, 106, etc.) are based on a unique numbering described in Lefranc et al. (2003) Dev. Comp. Immunol. 27:55-77, and can also be viewed on www.imgt.org.

[0082] In one embodiment, the human V_L gene segment is operably linked to a human or non-human leader sequence. In one embodiment, the leader sequence is a non-human leader sequence. In a specific embodiment, the non-human leader sequence is a mouse Vκ3-7 leader sequence. In a specific embodiment, the leader sequence is operably linked to an unrearranged human V_L gene segment. In a specific embodiment, the leader sequence is operably linked to a rearranged human V_L/J_L sequence. Thus, in one specific embodiment, the single rearranged Vκ1-39/Jκ5 or Vκ3-20/Jκ1 variable region gene sequence comprising at least one histidine substitution is operably linked to a mouse Vκ3-7 leader sequence.

[0083] In one embodiment, the V_L gene segment is operably linked to an immunoglobulin promoter sequence. In one embodiment, the promoter sequence is a human promoter sequence. In a specific embodiment, the human immunoglobulin promoter is a human Vκ3-15 promoter. In a specific embodiment, the promoter is operably linked to an unrearranged human V_L gene segment. In a specific embodiment, the promoter is operably linked to a rearranged human V_L/J_L sequence. Thus, in one specific embodiment, the single rearranged Vκ1-39/Jκ5 or Vκ3-20/Jκ1 variable region gene sequence comprising at least one histidine substitution is operably linked to the human Vκ3-15 promoter.

[0084] In one embodiment, the light chain locus comprises a leader sequence flanked 5' (with respect to transcriptional direction of a V_L gene segment) with a human immunoglobulin promoter and flanked 3' with a human V_L gene segment that rearranges with a human J_L segment and encodes a variable domain of a reverse chimeric light chain comprising an endogenous non-human light chain constant region (C_L). In a specific embodiment, the V_L and J_L gene segments are at the non-human Vκ locus, and the non-human C_L is a non-human Cκ (e.g., mouse Cκ). In one specific embodiment, the variable region sequence is operably linked to the non-human constant region sequence, e.g., the non-human Cκ gene sequence.

[0085] In one embodiment, the light chain locus comprises a leader sequence flanked 5' (with respect to transcriptional direction of a V_L gene segment) with a human immunoglobulin promoter and flanked 3' with a rearranged human variable region sequence (V_L/J_L sequence) and encodes a variable domain of a reverse chimeric light chain comprising an endogenous non-human light chain constant region (C_L). In a specific embodiment, the rearranged human V_L/J_L sequence is at the non-human kappa (κ) locus, and the non-human C_L is a non-human Cκ. In one specific embodiment, the rearranged human variable region sequence is operably linked to the non-human immunoglobulin light chain constant region sequence, e.g., the non-human Cκ gene sequence. In one embodiment, the non-human immunoglobulin light chain constant region sequence is an endogenous non-human sequence. In one embodiment, the non-human animal is a mouse and the Cκ gene sequence is a mouse Cκ gene sequence. In one embodiment, the rearranged human immunoglobulin light chain variable region sequence comprising a substitution of at least one non-histidine codon with a histidine codon is at the endogenous non-human (e.g., mouse) immunoglobulin light chain locus (κ locus). Exemplary embodiments of the locus are presented in FIGs. 8C, 8E, 14C, and 14D.

[0086] In one embodiment, the genetically modified non-human animal is a mouse, and the variable region locus of the mouse is a κ light chain locus, and the κ light chain locus

comprises a mouse κ intronic enhancer, a mouse κ 3' enhancer, or both an intronic enhancer and a 3' enhancer.

[0087] In one embodiment, the non-human animal (e.g., a rodent, e.g., a rat or a mouse) comprises a nonfunctional immunoglobulin lambda (λ) light chain locus. In a specific embodiment, the λ light chain locus comprises a deletion of one or more sequences of the locus, wherein the one or more deletions renders the λ light chain locus incapable of rearranging to form a light chain gene. In another embodiment, all or substantially all of the V_L gene segments of the λ light chain locus are deleted. In one embodiment, the non-human animal (e.g., rodent, e.g. mouse or rat) comprises a rearranged human immunoglobulin light chain variable region sequence comprising a substitution of at least one non-histidine codon with a histidine codon, and lacks a functional unrearranged immunoglobulin light chain variable region, e.g., endogenous unrearranged light chain variable region. In one embodiment, the rearranged, histidine-substituted human immunoglobulin light chain variable region gene sequence replaces endogenous unrearranged immunoglobulin light chain variable region gene sequence.

[0088] In one embodiment, the animal makes a light chain that comprises a somatically mutated variable domain derived from a human variable region sequence that comprises a substitution of at least one non-histidine codon with a histidine codon. In one embodiment, the light chain comprises a somatically mutated variable domain derived from a human variable region sequence that comprises a substitution of at least one non-histidine codon with a histidine codon, and a non-human Cκ region. In one embodiment, the non-human animal does not express a λ light chain.

[0089] One skilled in the art would appreciate that although substitution(s) of at least one non-histidine residue with a histidine residue is genetically engineered into the human immunoglobulin light chain variable region, due to somatic hypermutations, not all antibodies that are generated in the genetically modified non-human animal will harbor that histidine residue(s) at engineered position(s). However, generation of a wide repertoire of antibodies in the non-human animal will allow to select for *in vivo* generated antigen-specific antibodies that display high affinity for an antigen of interest while retaining histidine modifications introduced into the germline and, preferably, exhibiting pH-dependent antigen binding.

[0090] Thus, in one embodiment, the animal retains at least one histidine amino acid introduced by substitution of at least one non-histidine codon with a histidine codon in its variable region gene. In one embodiment, the animal retains all or substantially all histidine substitutions in its somatically mutated light chain variable domain that were introduced into its variable region gene.

[0091] In one embodiment, the genetically modified non-human animal described herein also comprises in its genome, e.g., in its germline, an unrearranged immunoglobulin heavy chain variable region comprising V_H, D_H, and J_H gene segment sequences. In one embodiment, the V_H, D_H, and J_H gene segment sequences are human V_H, D_H, and J_H gene segment sequences, and the unrearranged immunoglobulin heavy chain variable region is a human heavy chain variable region. In one embodiment, the human V_H, D_H, and J_H gene segment sequences are operably linked to non-human heavy chain constant region sequence. In one embodiment, the non-human heavy chain constant region sequence is an endogenous non-human heavy chain constant region sequence. In one embodiment, the human heavy chain gene segment sequences are at the endogenous non-human immunoglobulin heavy chain locus. In one embodiment, the human immunoglobulin heavy chain variable region sequence comprised in a non-human animal also comprises a substitution of at least one non-histidine codon for a histidine codon.

[0092] In one embodiment, the non-human animal described herein expresses an immunoglobulin light chain that comprises a non-human light chain constant region sequence. In one embodiment, the non-human animal expresses an immunoglobulin light chain that comprises a human light chain constant region sequence.

[0093] In one embodiment, the non-human animal described herein expresses an immunoglobulin heavy chain that comprises a non-human sequence selected from a C_H1 sequence, a hinge sequence, a C_H2 sequence, a C_H3 sequence, and a combination thereof.

[0094] In one embodiment, the non-human animal expresses an immunoglobulin heavy chain that comprises a human sequence selected from a C_H1 sequence, a hinge sequence, a C_H2 sequence, a C_H3 sequence, and a combination thereof.

[0095] In the embodiment where the animal comprises a single rearranged immunoglobulin light chain variable region comprising a substitution of at least one non-histidine codon with a histidine codon, the rearranged immunoglobulin light chain sequence in the germline of the animal is at an endogenous non-human immunoglobulin light chain locus. In a specific embodiment, the rearranged immunoglobulin light chain sequence comprising a substitution of at least one non-histidine codon with a histidine codon in the germline of the animal replaces all or substantially all endogenous non-human light chain V and J segment sequences at the endogenous non-human immunoglobulin light chain locus.

[0096] In one embodiment, the non-human animal comprises a replacement of endogenous V_H gene segments with one or more human V_H gene segments, wherein the human V_H gene segments are operably linked to a non-human C_H region gene, such that the non-human animal rearranges the human V_H gene segments and expresses a reverse

chimeric immunoglobulin heavy chain that comprises a human V_H domain and a non-human C_H. In one embodiment, 90-100% of unrearranged non-human V_H gene segments are replaced with at least one unrearranged human V_H gene segment. In a specific embodiment, all or substantially all (e.g., 90-100%) of the endogenous non-human V_H gene segments are replaced with at least one unrearranged human V_H gene segment. In one embodiment, the replacement is with at least 19, at least 39, or at least 80 or 81 unrearranged human V_H gene segments. In one embodiment, the replacement is with at least 12 functional unrearranged human V_H gene segments, at least 25 functional unrearranged human V_H gene segments, or at least 43 functional unrearranged human V_H gene segments. In one embodiment, the non-human animal comprises a replacement of all non-human D_H and J_H segments with at least one unrearranged human D_H segment and at least one unrearranged human J_H segment. In one embodiment, the non-human animal comprises a replacement of all non-human D_H and J_H segments with all unrearranged human D_H segments and all unrearranged human J_H segments.

[0097] A non-human animal, e.g., a mouse, comprising in its genome, e.g., in its germline, a limited repertoire of human immunoglobulin light chain variable regions, e.g., a single rearranged human immunoglobulin light chain variable region (e.g., Vκ1-39/Jκ5 or Vκ3-20/Jκ1), with a substitution of at least one non-histidine codon with a histidine codon and a diverse repertoire of unrearranged human V_H, D_H, and J_H segments is capable of generating antigen binding proteins encoded by heavy chain variable region sequences derived from various permutations of unrearranged human V_H, D_H, and J_H segments, wherein the V_H, D_H, and J_H segments present in the heavy chain variable sequences are derived from all or substantially all functional human V_H, D_H, and J_H segments present in the genome of the animal. Various available possibilities for heavy chain variable domain sequences expressed in the cells, e.g., B cells, of the genetically modified animals described herein (i.e., derived from combinations of various functional human V, D, and J segments) are described in U.S. Application Publication Nos. 2011/0195454, 2012/0021409, 2012/0192300 and 2013/0045492, all incorporated herein by reference. In various embodiments, the rearranged human immunoglobulin light chain variable region sequence comprising substitution(s) of at least one non-histidine codon with a histidine codon and the unrearranged human immunoglobulin heavy chain variable region sequence are comprised in the germline of the non-human animal.

[0098] In one embodiment, the non-human animal comprises one copy of one or both of the rearranged human immunoglobulin light chain variable region sequence comprising substitution(s) of at least one non-histidine codon with a histidine codon and the unrearranged human immunoglobulin heavy chain variable region sequence. In another

embodiment, the non-human animal comprises two copies of one or both of the rearranged human immunoglobulin light chain variable region sequence comprising substitution(s) of at least one non-histidine codon with a histidine codon and the unrearranged human immunoglobulin heavy chain variable region sequence. Thus, the non-human animal may be homozygous or heterozygous for one or both the rearranged human immunoglobulin light chain variable region sequence comprising substitution(s) of at least one non-histidine codon with a histidine codon and the unrearranged human immunoglobulin heavy chain variable region sequence.

[0099] In addition to genetically modified non-human animals comprising in their genome an immunoglobulin light chain variable region gene sequence (e.g., a single rearranged immunoglobulin light chain variable region gene sequence) comprising substitution of at least one non-histidine codon with a histidine codon (e.g., in CDR3 of the light chain), also provided herein are genetically modified non-human animals comprising an immunoglobulin light chain variable region gene sequence with one or more additions/insertions of histidine codon(s), such that the expressed variable domain comprises an additional amino acid(s) which, if not subject to somatic hypermutation, is a histidine.

[00100] The genetically modified non-human animal comprising a human immunoglobulin light chain variable region gene sequence with a substitution of at least one non-histidine codon with a histidine codon described herein may be selected from a group consisting of a mouse, rat, rabbit, pig, bovine (e.g., cow, bull, buffalo), deer, sheep, goat, chicken, cat, dog, ferret, primate (e.g., marmoset, rhesus monkey). For the non-human animals where suitable genetically modifiable ES cells are not readily available, methods distinct from those described herein 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 nuclear transfer to transfer the modified genome to a suitable cell, e.g., an oocyte, and gestating the modified cell (e.g., the modified oocyte) in a non-human animal under suitable conditions to form an embryo.

[00101] In one aspect, the non-human animal is a mammal. In one aspect, 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.

[00102] In a specific 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/6NJ, C57BL/10, C57BL/10ScSn, C57BL/10Cr, and C57BL/Ola. In another embodiment, the mouse is a 129 strain selected from the group consisting of a strain that is 129P1, 129P2, 129P3, 129X1, 129S1 (*e.g.*, 129S1/SV, 129S1/SvIm), 129S2, 129S4, 129S5, 129S9/SvEvH, 129S6 (129/SvEvTac), 129S7, 129S8, 129T1, 129T2 (see, *e.g.*, Festing *et al.* (1999) Revised nomenclature for strain 129 mice, Mammalian Genome 10:836, *see also*, Auerbach et al (2000) Establishment and Chimera Analysis of 129/SvEv- and C57BL/6-Derived Mouse Embryonic Stem Cell Lines). In a specific embodiment, the genetically modified mouse is a mix of an aforementioned 129 strain and an aforementioned C57BL/6 strain. In another specific embodiment, the mouse is a mix of aforementioned 129 strains, or a mix of aforementioned BL/6 strains. In a specific embodiment, the 129 strain of the mix is a 129S6 (129/SvEvTac) strain. In another embodiment, the mouse is a BALB strain, e.g., BALB/c strain. In yet another embodiment, the mouse is a mix of a BALB strain and another aforementioned strain.

[00103] 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 strains selected from the group consisting of Wistar, LEA, Sprague Dawley, Fischer, F344, F6, and Dark Agouti.

[00104] Thus, in one embodiment, the genetically modified non-human animal is a rodent. In one embodiment, the genetically modified non-human animal is a rat or a mouse. In one embodiment, the animal is a mouse. Thus, in one embodiment, provided herein is a genetically modified mouse comprising in its genome, e.g., in its germline, a single rearranged human immunoglobulin light chain variable region comprising human V_L and J_L gene sequences, wherein the single rearranged human immunoglobulin light chain variable region comprises a substitution of at least non-histidine codon with a histidine codon. In one embodiment, the mouse lacks a functional unrearranged immunoglobulin light chain variable region (e.g., lacks functional unrearranged V and J gene segment sequences). In one

embodiment, the rearranged human immunoglobulin light chain variable region with histidine codon substitution(s) is Vκ1-39/Jκ or Vκ3-20/Jκ variable region. In one embodiment the J segment sequence is selected from Jκ1, Jκ2, Jκ3, Jκ4, and Jκ5. In one embodiment the J segment sequence is Jκ1 or Jκ5. In one embodiment, the substitution of at least one non-histidine codon with a histidine codon is in the nucleotide sequence encoding a CDR3 region. In one embodiment, wherein the rearranged variable region sequence is Vκ1-39/Jκ5 sequence, the histidine substitution(s) is designed to express at a position selected from 105, 106, 108, 111, and a combination thereof. In another embodiment, wherein the rearranged variable region sequence is Vκ3-20/Jκ1 sequence, the histidine substitution(s) is designed to express at a position selected from 105, 106, 107, 109, and a combination thereof. In one embodiment, the rearranged immunoglobulin light chain variable region with substituted histidine codon(s) is operably linked to an endogenous mouse immunoglobulin constant region gene sequence (e.g., Cκ gene sequence). In one embodiment, the mouse further comprises in its genome, e.g., in its germline, an unrearranged immunoglobulin heavy chain variable region comprising human V_H, D_H, and J_H segments. In one embodiment, human V_H, D_H, and J_H segments are operably linked to an endogenous mouse immunoglobulin heavy chain constant region gene sequence. In various embodiments, the rearranged human immunoglobulin light chain variable region sequence comprising substitution(s) of at least one non-histidine codon with a histidine codon and the unrearranged human immunoglobulin heavy chain variable region sequence are comprised in the germline of the mouse.

[00105] Also provided herein are targeting vectors for generating genetically modified non-human animals, e.g., mice, described herein. In one aspect, provided is a targeting vector comprising, from 5' to 3' in transcriptional direction with reference to the sequences of the 5' and 3' mouse homology arms of the vector, a 5' mouse homology arm, a human or mouse immunoglobulin promoter, a human or mouse leader sequence, a human variable region selected from a rearranged human Vκ1-39Jκ5 or a rearranged human Vκ3-20Jκ1 and comprising a substitution of at least one non-histidine codon with a histidine codon, and a 3' mouse homology arm. In one embodiment, the 5' and 3' homology arms target the vector to a sequence 5' with respect to an enhancer sequence that is present 5' and proximal to the mouse Cκ gene. In another embodiment, the targeting vector comprises a 5' mouse homology arm followed by a selection cassette flanked by recombination sites, human or mouse immunoglobulin promoter, human or mouse leader sequence, a human variable region selected from a rearranged human Vκ1-39Jκ5 or a rearranged human Vκ3-20Jκ1 and comprising a substitution of at least one non-histidine codon with a histidine

codon, followed by the 3' mouse homology arm that comprises mouse enhancers and constant region (Cκ) sequences.

[00106] A selection cassette is a nucleotide sequence inserted into a targeting construct to facilitate selection of cells (e.g., ES cells) that have integrated the construct of interest. A number of suitable selection cassettes are known in the art. Commonly, a selection cassette enables positive selection in the presence of a particular antibiotic (e.g., Neo, Hyg, Pur, CM, Spec, etc.). In addition, a selection cassette may be flanked by recombination sites, which allow deletion of the selection cassette upon treatment with recombinase enzymes. Commonly used recombination sites are *lox*P and *Frt*, recognized by Cre and Flp enzymes, respectively, but others are known in the art.

[00107] In one embodiment, the promoter is a human immunoglobulin variable region gene segment promoter. In a specific embodiment, the promoter is a human Vκ3-15 promoter. In one embodiment, the leader sequence is a mouse leader sequence. In a specific embodiment, the mouse leader sequence is a mouse Vκ3-7 leader sequence. Exemplary embodiments of the targeting vectors are presented in FIGs. 8B and 14B.

[00108] In one aspect, a targeting vector is provided as described above, but in place of the 5' mouse homology arm the human or mouse promoter is flanked 5' with a site-specific recombinase recognition site (SRRS), and in place of the 3' mouse homology arm the human V_L region is flanked 3' with an SRRS.

[00109] Also provided herein are methods of making genetically modified non-human animals (e.g., rodents, e.g., mice or rats) described herein. In one aspect, the method for making a genetically modified non-human animal described herein utilizes a targeting vector, made using VELOCIGENE® technology, introducing the construct into ES cells, and introducing targeted ES cell clones into a mouse embryo using VELOCIMOUSE® technology, as described in the Examples. Histidine modifications may be introduced into the targeting vector using a variety of molecular biology techniques, e.g., site directed mutagenesis or de novo DNA synthesis. Upon completion of gene targeting, ES cells of genetically modified non-human animals are screened to confirm successful incorporation of exogenous nucleotide sequence of interest or expression of exogenous polypeptide. Numerous techniques are known to those skilled in the art, and include (but are not limited to) Southern blotting, long PCR, quantitative PCT (e.g., real-time PCR using TAQMAN®), fluorescence *in situ* hybridization, Northern blotting, flow cytometry, Western analysis, immunocytochemistry, immunohistochemistry, etc. In one example, non-human animals (e.g., mice) bearing the genetic modification of interest can be identified by screening for loss of mouse allele and/or gain of human allele using a modification of allele assay

described in Valenzuela *et al.* (2003) High-throughput engineering of the mouse genome coupled with high-resolution expression analysis, Nature Biotech. 21(6):652-659. Other assays that identify a specific nucleotide or amino acid sequence in the genetically modified animals are known to those skilled in the art.

[00110] Thus, in one embodiment, the method of generating genetically modified non-human animals comprises replacing an immunoglobulin light chain variable region gene sequence in the animal with a human immunoglobulin light chain variable region gene sequence (comprising human V_L and J_L gene segments) wherein the human immunoglobulin variable region gene sequence comprises a substitution of at least one non-histidine codon with a histidine codon. In one embodiment, the substitution of at least one non-histidine codon with a histidine codon is in the nucleotide sequence encoding a CDR region, e.g., a CDR3 region.

[00111] In one embodiment, the method of generating genetically modified non-human animals described herein comprises replacing an immunoglobulin light chain variable region gene sequence in the animal with a single rearranged human immunoglobulin light chain variable region gene sequence comprising human V_L and J_L gene segment sequences, wherein the single rearranged human immunoglobulin variable region gene sequence comprises a substitution of at least one non-histidine codon with a histidine codon. In one embodiment, the substitution is in a CDR codon. In one embodiment, the substitution is of one, two, three, four, or more CDR3 codon(s). In one embodiment, the single rearranged human immunoglobulin light chain variable region gene sequence is based on the human germline rearranged light chain variable region sequence selected from Vκ1-39Jκ5 and Vκ3-20Jκ1. Thus, in one embodiment, where the single rearranged human immunoglobulin light chain variable region gene sequence is derived from Vκ1-39Jκ5, replacement of at least one non-histidine codon with histidine codon is designed to express a histidine at positions selected from 105, 106, 108, 111, and a combination thereof. In one embodiment, where the single rearranged human immunoglobulin light chain variable region gene sequence is derived from Vκ3-20κ1, replacement of at least one non-histidine codon with a histidine codon is designed to express a histidine at position selected from 105, 106, 107, 109, and a combination thereof.

[00112] In another embodiment, the method of generating a non-human animal described herein (i.e., comprising a genetically modified immunoglobulin light chain locus described herein) comprises modifying a genome of a non-human animal to delete or render non-functional endogenous immunoglobulin light chain V and J segments in an immunoglobulin light chain locus, and placing in the genome a single rearranged human light chain variable

region gene sequence comprising a substitution of at least one non-histidine codon with a histidine codon. In one embodiment, the method results in a genetically modified non-human animal that comprises a population of B cells enriched for antibodies exhibiting pH dependent binding to an antigen of interest.

[00113] In some embodiments, the methods of generating genetically modified non-human animals described herein comprise replacing an immunoglobulin light chain variable region gene sequence with human immunoglobulin light chain variable gene region sequence comprising substitution(s) of at least one non-histidine codon with a histidine codon in the animal that also comprises a replacement of endogenous non-human immunoglobulin heavy chain variable region gene sequence with a human immunoglobulin heavy chain variable region gene sequence comprising at least one of each or a repertoire of human V_H, D_H, and J_H sequences, as described above. In one embodiment, in order to generate a non-human animal comprising a replacement of endogenous immunoglobulin light chain variable region gene sequence human light chain variable region gene sequence comprising a substitution of at least one non-histidine codon with a histidine codon and a replacement of endogenous non-human immunoglobulin heavy chain variable region gene sequence with a human immunoglobulin heavy chain variable region gene sequence, the animal with replacement of light chain variable region gene sequence is bred to an animal with replacement of heavy chain variable region gene sequence.

[00114] Inventors presently provide genetically engineered non-human animals (e.g., rodents, e.g., rats or mice) that express antigen-binding proteins, e.g., antibodies, that comprise a universal light chain, e.g., a human universal light chain (e.g., a light chain derived from a single rearranged human immunoglobulin light chain variable region) that comprises one or more histidine modifications, wherein the antigen-binding proteins exhibit a pH-dependent antigen binding of a target antigen. The animals are genetically engineered to include a light chain CDR3 that comprises one or more histidine modifications. In various embodiments, the light chain CDR3 comprises two, three, or four or more histidine residues in a cluster.

[00115] In one embodiment, provided herein is a genetically engineered non-human animal (e.g., a mouse or a rat) that comprises a population of B cells characterized by enhanced presence of histidines in immunoglobulin light chains, e.g., immunoglobulin variable domains, e.g., immunoglobulin CDRs, compared to a wild type animal. In one embodiment, enhancement of histidine presence is about 2 to 4 fold. In one embodiment, enhancement of histidines is about 2 to 10 fold.

[00116] In one embodiment, provided herein is a genetically engineered non-human animal that comprises a population of antigen-specific antibodies that express histidine residue(s) as a result of codon modifications in the light chain variable region gene sequence, and display pH-dependent binding of target antigen. In one embodiment, these animals comprise a population of B cells that are enriched for antibodies, e.g., antigen-specific antibodies, that display pH-dependent binding properties (e.g., decreased dissociative half-life ($t_{1/2}$), at acidic pH vs neutral pH) as compared to a population of antigen-specific antibodies generated in animals that do not comprise a substitution of at least one non-histidine codon with a histidine codon in immunoglobulin light chain variable region described herein. In one embodiment, the enrichment of antigen-specific antibodies displaying pH-dependent antigen binding properties generated in the genetically engineered animals described herein as compared to similar animals that do comprise histidine substitutions in light chain variable region is greater than about 2 fold, e.g., greater than about 5 fold, e.g., greater than about 10 fold. Thus, the genetically modified animals of the invention are enriched for antibodies with improved antibody recycling properties, which is desired in order to reduce target-mediated clearance as well as to reduce the dose and/or dosing frequency of a therapeutic antigen-binding protein developed based on such *in vivo* generated antibody format.

[00117] Thus, provided herein is an antigen-binding protein, generated in genetically modified non-human animals described herein, wherein the antigen-binding protein displays pH-dependent antigen binding. In one embodiment, the antigen-binding protein is an antibody, e.g., antigen-specific antibody. In one embodiment, the antibody comprises a light chain which comprises a human light chain variable domain derived from a rearrangement of human immunoglobulin light chain variable gene segments where at least one non-histidine codon was substituted for a histidine codon in the germline gene sequence, and wherein the antibody retains at least one histidine substitution in its expressed human light chain variable domain. In one embodiment, the antibody comprises a light chain which comprises a human light chain variable domain derived from a single rearranged human light chain variable region gene sequence, wherein the single rearranged light chain variable region gene sequence comprises a substitution of at least one non-histidine codon with a histidine codon, and wherein the antibody retains at least one histidine substitution in its expressed light chain variable domain. In one embodiment, the antibody comprises a light chain derived from a human Vκ1-39Jκ5 or Vκ3-20Jκ1 rearrangement, wherein the human Vκ1-39Jκ5 or Vκ3-20Jκ1 gene sequence comprises a substitution of at least one non-histidine codon with a histidine codon, and wherein the antibody retains at least one histidine substitution in its expressed light chain variable domain. In some embodiments, the antibody retains all or

substantially all histidine substitutions in its expressed light chain variable domain. In one embodiment, the substitution is of three non-histidine codons with three histidine codons in the nucleotide sequence encoding CDR3 of the light chain variable region gene sequence, and the antibody retains all three histidine substitutions in its expressed light chain variable domain. In one embodiment, the substitution is of four non-histidine codons with four histidine codons in the nucleotide sequence encoding CDR3 of the light chain variable region gene sequence, and the antibody retains three or four histidine substitutions in its expressed light chain variable domain.

[00118] In one embodiment, the light chain of the antibody further comprises a non-human light chain constant region amino acid sequence, e.g., endogenous light chain constant region amino acid sequence. In addition, the antibody, e.g., antigen-specific antibody, generated in a genetically modified non-human animal described herein also comprises a heavy chain which comprises a human heavy chain variable domain derived from a rearrangement of human heavy chain V, D, and J segments. Human heavy chain V, D, and J segments may be selected from a repertoire of human heavy chain segments present at the endogenous non-human heavy chain locus, e.g., at least one functional V, at least one functional D, and at least one functional J segment, e.g., up to a complete repertoire of functional human V, D, and J segments. Exemplary possible rearrangements of human heavy chain variable segments may be gleaned from a listing of functional human V, D, and J segments in IMGT database, and from U.S. Application Publication Nos. 2011/0195454, 2012/0021409, 2012/0192309, and 2013/0045492, incorporated herein by reference. Furthermore, in one embodiment, the heavy chain of the antibody comprises a non-human heavy chain constant region amino acid sequence, e.g., an endogenous non-human heavy chain constant region amino acid sequence. In one embodiment, the non-human heavy chain constant region comprises C_H1, hinge, C_H2, and C_H3 domains. In one embodiment, the antibody is an IgG, IgE, IgD, IgM, or IgA isotype.

[00119] Thus, in one embodiment, provided herein is a binding protein generated in the genetically modified non-human animals described herein, wherein the binding protein comprises a reverse chimeric light chain comprising (a) a light chain variable domain derived from a human Vκ1-39Jκ5 rearrangement comprising a substitution of at least one non-histidine codon with a histidine codon, wherein the light chain retains at least one histidine substitution in its expressed light chain variable domain and (b) a non-human, e.g., a mouse, light chain constant region amino acid sequence, wherein the light chain is associated with a reverse chimeric heavy chain comprising (a) a heavy chain variable domain derived from a rearrangement of human V, D, and J segments, wherein the V, D, and J segments are selected from a repertoire of human V, D, and J segments present in the animal, and (b) a

non-human, e.g., mouse, heavy chain constant region amino acid sequence. In one embodiment, the repertoire of human V, D, and J segments comprises at least one functional V, at least one functional D, and at least one functional J segment, e.g., up to a complete repertoire of functional human V, D, and J segments. In one embodiment, the heavy and the light chain constant domains are endogenous heavy and light chain constant regions. In one embodiment, the heavy and light chain variable domains are somatically mutated domains. In one embodiment, the somatically mutated light chain domain retains at least one histidine substitution introduced into the germline sequence. In some embodiments, the somatically mutated light chain domain retains all or substantially all histidine substitutions introduced into the germline sequence. In one embodiment, the antigen-binding protein displays pH-dependent antigen binding properties.

[00120] In another embodiment, provided herein is a binding protein generated in the genetically modified non-human animals described herein, wherein the binding protein comprises a reverse chimeric light chain comprising (a) a light chain variable domain derived from a human Vκ3-20Jκ1 rearrangement comprising a substitution of at least one non-histidine codon with a histidine codon, wherein the light chain retains at least one histidine substitution in its expressed light chain variable domain and (b) a non-human, e.g., a mouse, light chain constant region amino acid sequence, wherein the light chain is associated with a reverse chimeric heavy chain comprising (a) a heavy chain variable domain derived from a rearrangement of human V, D, and J segments, wherein the V, D, and J segments are selected from a repertoire of human V, D, and J segments present in the animal, and (b) a non-human, e.g., mouse, heavy chain constant region amino acid sequence. In one embodiment, the repertoire of human V, D, and J segments comprises at least one functional V, at least one functional D, and at least one functional J segment, e.g., up to a complete repertoire of functional human V, D, and J segments. In one embodiment, the heavy and the light chain constant regions are endogenous heavy and light chain constant regions. In one embodiment, the heavy and light chain variable domains are somatically mutated domains. In one embodiment, the somatically mutated light chain domain retains at least one histidine substitution introduced into the germline sequence. In some embodiments, the somatically mutated light chain domain retains all or substantially all histidine substitutions introduced into the germline sequence. In one embodiment, the antigen-binding protein displays pH-dependent antigen binding properties.

[00121] In one embodiment, also provided herein is a B cell of the genetically modified animal described herein, that comprises in its germline a histidine-modified human light chain variable region sequence, e.g., a histidine-modified single rearranged human light chain variable region sequence, described herein, and expresses an antigen-binding protein

described herein. In one embodiment, the antigen-binding protein, e.g., an antibody, expressed in the B cell retains at least one histidine residue introduced into the germline, and displays pH-dependent antigen-binding properties. In some embodiments, the antigen-binding protein, e.g., an antibody, expressed in the B cell retains all or substantially all histidine residues introduced into the germline, and displays pH-dependent antigen-binding properties.

[00122] In various embodiments, the genetically modified non-human animal described herein comprises a human light chain variable region gene sequence, e.g., a single rearranged human light chain variable region gene sequence (e.g., Vκ1-39Jκ5 or Vκ3-20Jκ1 sequence) that comprises a substitution of at least one non-histidine codon with a histidine codon (or an addition of a histidine codon into the germline sequence). These additions or substitutions result in a non-human animal that comprises a population of B cells enriched for antigen-binding proteins with pH dependent binding properties for their antigens. In one embodiment, antigen-binding proteins, e.g., antibodies, generated in the non-human animals described herein in response to antigen stimulation display pH dependent antigen binding while exhibiting high affinity for the antigen at neutral pH, e.g., pH between about 7.0 and about 8.0, e.g., pH between about 7.0 and about 7.4, e.g., between about 7.2 and about 7.4, e.g., physiological pH. In one embodiment, the affinity of the antigen-binding protein to its antigen, expressed as a dissociation constant (K_D) at a neutral pH is less than 10^{-6} M, e.g., less than 10^{-8} M, e.g., less than 10^{-9} M, e.g., less than 10^{-10} M, e.g., less than 10^{-11} M, e.g., less than 10^{-12} M.

[00123] In one embodiment, an antigen-binding protein, e.g., an antibody, generated in the genetically modified non-human animal described herein, exhibits reduced binding to its antigen in acidic pH (e.g., pH of 6.0 or lower, e.g., pH between about 5.0 and about 6.0, pH between about 5.75 and about 6.0, e.g., pH of endosomal or lysosomal compartments) as compared to neutral pH. In one embodiment, the antigen-binding protein, e.g., the antibody, generated in the genetically modified non-human animal described herein, exhibits no binding to the antigen in acidic pH, while retaining binding to the antigen at neutral pH. In one embodiment, an antigen-binding protein generated by the genetically modified non-human animal described herein, has a decrease in dissociative half-life ($t_{1/2}$) at an acidic pH as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH of 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. In one embodiment, an antigen-binding protein expressed by the genetically modified non-human animal described herein has a $t_{1/2}$ at an acidic pH and 37°C of about 2 min or less. In one embodiment, an antigen-binding protein expressed by the genetically

modified non-human animal described herein has a $t_{1/2}$ at an acidic pH and 37°C of less than about 1 min. In one embodiment, an antigen-binding protein expressed by the genetically modified non-human animal described herein has a $t_{1/2}$ at an acidic pH and 25°C of about 2 min or less. In one embodiment, an antigen-binding protein expressed by the genetically modified non-human animal described herein has a $t_{1/2}$ at an acidic pH and 25°C of less than about 1 min.

[00124] Kinetic parameters, such as equilibrium dissociation constants (K_D) and dissociative half-lives ($t_{½}$) can be calculated from kinetic rate constant as: K_D (M) = k_d / k_a; and $t_{½}$ (min) = ln2/(60*k_d).

[00125] In one embodiment, the antigen-binding protein, e.g., an antibody, generated in the genetically modified non-human animals described herein, exhibits increased binding to FcRn molecule. As described above, FcRn is a receptor present inside the endosomal compartment that is capable of binding immunoglobulins at an acidic pH and recycling them back to the surface. Screening antibody molecules in the genetically modified non-human animals described herein presents a unique opportunity to select for antibodies with three beneficial parameters: high affinity for an antigen, pH-dependent antigen binding (with weaker antigen binding at acidic pH) and increased binding to FcRn.

[00126] In one embodiment, a genetically modified non-human animal described herein comprises a population of B cells in response to an antigen that produces and is enriched for antigen-binding proteins, e.g., antibodies, that, when reformatted into therapeutics, exhibit increased serum half life upon administration of a therapeutic dose to a subject over an equivalent B cell population produced in response to the same antigen in non-human animals that do not comprise histidine modification(s) in their human light chain variable region gene sequences. Thus, in one embodiment, an antigen-binding protein, e.g., an antibody, produced in response to an antigen of interest in a genetically modified non-human animal described herein, when reformatted into a therapeutic, exhibits increased serum half life upon administration of a therapeutic dose to a subject over a serum half life of an antigen-binding protein (when reformatted into a therapeutic and administered at the same therapeutic dose) that was produced in response to the same antigen in a non-human animal that does not comprise histidine modification(s) in its human light chain variable region gene sequence. In some embodiments, the increase in serum half life is about 2 fold, e.g., about 5 fold, e.g., about 10 fold, e.g., about 15 fold, e.g., about 20 fold, or greater.

[00127] In one aspect, a pluripotent, induced pluripotent, or totipotent cell derived from a non-human as described herein is provided. In a specific embodiment, the cell is an embryonic stem (ES) cell.

[00128] In one aspect, a tissue derived from a non-human animal as described herein is provided. In one embodiment, the tissue is derived from spleen, lymph node or bone marrow of a non-human animal as described herein.

[00129] In one aspect, a nucleus derived from a non-human animal as described herein is provided. In one embodiment, the nucleus is from a diploid cell that is not a B cell.

[00130] In one aspect, a non-human cell is provided that is isolated from a non-human animal (e.g., a rodent, e.g., a mouse or a rat) as described herein. In one embodiment, the cell is an ES cell. In one embodiment, the cell is a lymphocyte. In one embodiment, the lymphocyte is a B cell. In one embodiment, the B cell expresses a chimeric heavy chain comprising a variable domain derived from a human gene segment; and a light chain derived from a rearranged human Vκ1-39/J sequence with a substitution of at least one non-histidine codon with histidine codon, rearranged human Vκ3-20/J sequence with a substitution of at least one non-histidine codon with histidine codon, or a combination thereof and further comprising a substitution of at least one amino acid encoded in the germline for a histidine; wherein the heavy chain variable domain is fused to a non-human or a human heavy chain constant region and the light chain variable domain is fused to a non-human or a human light chain constant region.

[00131] In one aspect, a hybridoma is provided, wherein the hybridoma is made with a B cell of a non-human animal as described herein. In a specific embodiment, the B cell is from a mouse as described herein that has been immunized with an immunogen comprising an epitope of interest, and the B cell expresses a binding protein that binds the epitope of interest, the binding protein has a somatically mutated human variable heavy chain domain and a mouse C_H, and has a human variable light chain domain derived from a rearranged human Vκ1-39Jκ5 with a substitution of at least one non-histidine codon with histidine codon or a rearranged human Vκ3-20Jκ1 with a substitution of at least one non-histidine codon with histidine codon and a mouse C_L, wherein the human light chain domain comprises a substitution of at least one amino acid encoded in the germline with a histidine.

[00132] Also provided is a cell expressing an antigen-binding protein generated in the non-human animals described herein. In one embodiment, the cell is selected from CHO, COS, 293, HeLa, and a retinal cell expressing a viral nucleic acid sequence (*e.g.,* a PERC.6™ cell).

[00133] In one aspect, a non-human embryo is provided, wherein the embryo comprises a donor ES cell that is derived from a non-human animal as described herein.

[00134] The non-human animals described herein are useful to generate B cells that express antibodies having histidines in a CDR3. An animal that places histidines in a CDR3 is useful for making antibodies in general, and in particular useful for developing antibodies that bind a target with sufficient affinity at or around a neutral pH, but that either do not bind or that bind weaker to the same target at an acidic pH.

[00135] The non-human animal is useful to generate variable regions of antibodies that can be used to make, e.g., human therapeutic binding proteins that bind their targets by human immunoglobulin variable domains that comprise the histidines in a CDR3. The altered binding at a lower pH will in some circumstances allow faster turnover because the therapeutic will bind a target on a cell's surface, be internalized in 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 (*e.g.,* on another cell or the same cell). In some circumstances, this will result in the ability to dose the therapeutic at a lower dose, or dose the therapeutic less frequently. This is particularly useful where it is not desirable to dose frequently, or to administer above a certain dosage, for safety or toxicity reasons. As a result, the serum half life of the antibody therapeutic when administered to a subject will be increased.

[00136] The non-human animal, e.g., rodent, e.g., mouse or rat, is useful in a method for increasing the number of B cells in an animal that exhibit an antibody variable region having a CDR3 with one or more histidines in it. The non-human animal is useful for generating antibody sequences that will exhibit pH-dependent antigen binding. The non-human animal is useful for generating a greater number of antibody sequences, resulting from a single immunization, wherein the antibodies will exhibit a pH-dependent antigen binding.

Antigen-Binding Proteins and Methods of Generating the Same

[00137] In one aspect, also provided herein are methods for generating human antigen-binding proteins, e.g., antibodies, which exhibit pH-dependent antigen binding, from the genetically modified non-human animals described herein with standard methods used in the art.

[00138] Several techniques for producing antibodies have been described. For example, in various embodiments chimeric antibodies are produced in mice as described herein. Antibodies can be isolated directly from B cells of an immunized mouse (*e.g.*, see U.S. 2007/0280945A1) and/or the B cells of the immunized mouse can be used to make hybridomas (Kohler and Milstein, 1975, *Nature* 256:495-497). DNA encoding the antibodies (human heavy and/or light chains) from non-human animals as described herein is readily isolated and sequenced using conventional techniques. Hybridoma and/or B cells derived

from non-human animals as described herein serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the non-human sequences. Thus, once nucleic acid sequences of antibodies with desired characteristics, e.g., affinity, epitope, pH-dependent antigen binding, etc., are determined, the non-human constant region gene sequences are replaced with a desired human constant region sequences to generate a fully human antibody containing a non-IgM isotype, for example, IgG1, IgG2, IgG3 or IgG4.

[00139] Thus, in one embodiment provided herein is a method of generating an antibody that exhibits pH-dependent antigen binding properties comprising generating a non-human animal (e.g., a mouse) as described herein, immunizing a mouse with an antigen of interest, allowing a non-human animal to mount an immune response to the antigen, and selecting in the non-human animal an antigen-specific antibody that exhibits pH dependent antigen binding properties, e.g., weaker binding to the antigen at an acidic than at neutral pH.

[00140] Also provided herein are methods of making multi-specific antigen binding proteins, e.g., bispecific antigen-binding proteins. These are molecules capable of binding more than one epitope with high affinity. Advantages of the invention include the ability to select suitably high binding (*e.g.,* affinity matured) heavy chain immunoglobulin chains each of which will associate with a single light chain. In addition, advantages of the invention include the ability to generate a multi-specific, e.g., a bispecific, antigen-binding protein that exhibits pH-dependent antigen binding.

[00141] Because of the dual nature of bispecific antibodies (*i.e.*, may be specific for different epitopes of one polypeptide or may contain antigen-binding domains specific for more than one target polypeptide, see, *e.g.*, Tutt *et al.*, 1991, *J. Immunol.* 147:60-69; Kufer *et al.*, 2004, *Trends Biotechnol.* 22:238-244), they offer many useful advantages for therapeutic application. For example, the bispecific antibodies can be used for redirected cytotoxicity (*e.g.*, to kill tumor cells), as a vaccine adjuvant, for delivering thrombolytic agents to clots, for converting enzyme activated prodrugs at a target site (*e.g.*, a tumor), for treating infectious diseases, targeting immune complexes to cell surface receptors, or for delivering immunotoxins to tumor cells.

[00142] The bispecific antibodies described herein can also be used in several therapeutic and non-therapeutic and/or diagnostic assay methods, such as, enzyme immunoassays, two-site immunoassays, *in vitro* or *in vivo* immunodiagnosis of various

diseases (*e.g.*, cancer), competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Other uses for the bispecific antibodies will be apparent to those skilled in the art.

[00143] Several techniques for making bispecific antibody fragments from recombinant cell culture have been reported. However, synthesis and expression of bispecific binding proteins has been problematic, in part due to issues associated with identifying a suitable light chain that can associate and express with two different heavy chains, and in part due to isolation issues. In various embodiments, compositions and methods described herein provide the advantage of full length bispecific antibodies that do not require special modification(s) to maintain traditional immunoglobulin structure by increasing stability/interaction of the components. In various embodiments, such modification(s) has proven cumbersome and served as an obstacle to development of bispecific antibody technology and their potential use in treating for human disease. Thus, in various embodiments, through providing a natural immunoglobulin structure (*i.e.,* full length) having the added property of multiple specificities, full length bispecific antibodies maintain their critical effector functions that previous bispecific fragments lacked, and further provide therapeutics that demonstrate the important pharmacokinetic parameter of a longer half-life.

[00144] Methods and compositions described herein allow for a genetically modified mouse to select, through otherwise natural processes, a suitable light chain that can associate and express with more than one heavy chain, including heavy chains that are somatically mutated (*e.g.,* affinity matured), wherein the light chain further confers upon the antigen-binding protein its pH-dependent antigen binding property. Human heavy and light chain variable region sequences from suitable B cells of immunized mice as described herein that express affinity matured antibodies having reverse chimeric heavy chains (i.e., human variable and mouse constant) can be identified and cloned in frame in an expression vector with a suitable human constant region gene sequence (*e.g.,* a human IgG1). Two such constructs can be prepared, wherein each construct encodes a human heavy chain variable domain that binds a different epitope. One of the human light chain variable regions (*e.g.,* human Vκ1-39Jκ5 or human Vκ3-20Jκ1), comprising a substitution of at least one non-histidine codon with a histidine codon, can be fused in frame to a suitable human light chain constant region gene (*e.g.,* a human κ constant gene). These three fully human heavy and light constructs can be placed in a suitable cell for expression. The cell will express two major species: a homodimeric heavy chain with the identical light chain, and a heterodimeric heavy chain with the identical light chain. To allow for a facile separation of these major species, one of the heavy chains is modified to omit a Protein A-binding determinant, resulting in a differential affinity of a homodimeric binding protein from a

heterodimeric binding protein. Compositions and methods that address this issue are described in USSN 12/832,838, filed 25 June 2010, entitled “Readily Isolated Bispecific Antibodies with Native Immunoglobulin Format,” published as US 2010/0331527A1, hereby incorporated by reference. Once the specie comprising heterodimeric heavy chain with an identical light chain is selected, this bi-specific antigen binding protein can be screened to confirm the retention of its pH-dependent antigen binding property.

[00145] In one aspect, an epitope-binding protein as described herein is provided, wherein human light chain and heavy chain variable region sequences are derived from animals described herein that have been immunized with an antigen comprising an epitope of interest.

[00146] In one embodiment, an epitope-binding protein is provided that comprises a first and a second polypeptide, the first polypeptide comprising, from N-terminal to C-terminal, a first epitope-binding region that selectively binds a first epitope, followed by a constant region that comprises a first C_H3 region of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and, a second polypeptide comprising, from N-terminal to C-terminal, a second epitope-binding region that selectively binds a second epitope, followed by a constant region that comprises a second C_H3 region of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, wherein the second C_H3 region comprises a modification that reduces or eliminates binding of the second C_H3 domain to protein A. Various such modifications are described in, e.g., U.S. Application Publication Nos. 2010/0331527 and 2011/0195454, incorporated herein by reference.

[00147] One method for making an epitope-binding protein that binds more than one epitope and exhibits pH-dependent epitope binding property is to immunize a first mouse in accordance with the invention with an antigen that comprises a first epitope of interest, wherein the mouse comprises (1) an endogenous immunoglobulin light chain variable region locus that does not contain an endogenous mouse light chain variable region gene sequence that is capable of rearranging and forming a light chain, wherein at the endogenous mouse immunoglobulin light chain variable region locus is a single rearranged human light chain variable region operably linked to the mouse endogenous light chain constant region gene, and the rearranged human light chain variable region is selected from a human Vκ1-39Jκ5 and a human Vκ3-20Jκ1 comprising a substitution of at least one non-histidine codon with a histidine condon, and (2) the endogenous mouse V_H gene segments have been replaced in whole or in part with human V_H gene segments, such that immunoglobulin heavy chains made by the mouse are solely or substantially heavy chains that comprise human variable domains and mouse constant domains. When immunized, such a mouse will make a reverse chimeric antibody, comprising only one of two human light

chain variable domains (*e.g.,* one of human Vκ1-39Jκ5 or human Vκ3-20Jκ1, e.g., comprising a substitution of at least one amino acid with a histidine). Commonly, at least some of the substituted histidine residues introduced into the germline sequence will be retained in the reverse chimeric antibody. Once a B cell is identified that encodes a heavy chain variable domain that binds the epitope of interest and expresses an antibody that exhibits pH-dependent antigen binding properties, the nucleotide sequence of the heavy chain variable region (and, optionally, the light chain variable region) can be retrieved (*e.g.,* by PCR) and cloned into an expression construct in frame with a suitable human immunoglobulin heavy chain constant region sequence. This process can be repeated to identify a second heavy chain variable domain that binds a second epitope, and a second heavy chain variable region gene sequence can be retrieved and cloned into an expression vector in frame to a second suitable human immunoglobulin heavy chain constant region sequence. The first and the second immunoglobulin constant domains encoded by the constant region gene sequence can be the same or different isotype, and one of the immunoglobulin constant domains (but not the other) can be modified as described herein or in US 2010/0331527A1, and epitope-binding protein can be expressed in a suitable cell and isolated based on its differential affinity for Protein A as compared to a homodimeric epitope-binding protein, *e.g.,* as described in US 2010/0331527A1.

[00148] Thus, in various embodiments, following isolation of the DNA and selection of the first and second nucleic acid sequences that encode the first and second human heavy chain variable domains having the desired specificities/affinities, and a third nucleic acid sequence that encodes a human light chain domain (a germline rearranged sequence or a light chain sequence isolated from a non-human animal as described herein) and comprises a substitution of at least one non-histidine codon with a histidine codon, the three nucleic acids sequences encoding the molecules are expressed to form the bispecific antibody using recombinant techniques which are widely available in the art. Often, the expression system of choice will involve a mammalian cell expression vector and host so that the bispecific antibody is appropriately glycosylated (*e.g.*, in the case of bispecific antibodies comprising antibody domains which are glycosylated). However, the molecules can also be produced in the prokaryotic expression systems. Normally, the host cell will be transformed with DNA encoding both the first human heavy chain variable domain, the second human heavy chain variable domain, the human light chain domain on a single vector or independent vectors. However, it is possible to express the first human heavy chain variable domain, second human heavy chain variable domain, and human light chain domain (the bispecific antibody components) in independent expression systems and couple the expressed polypeptides in vitro. In various embodiments, the human light chain domain is

derived from a germline sequence but for the substitution of at least one non-histidine codon with a histidine codon, e.g., in a CDR codon. In various embodiments, the human light chain domain comprises no more than one, no more than two, no more than three, no more than four, or no more than five somatic hypermutations within the light chain variable sequence of the light chain domain. In some embodiments, the somatic hypermutations do not alter the presence of at least one histidine residue introduced into the germline sequence of the light chain variable region.

[00149] In various embodiments, the nucleic acid(s) (*e.g.*, cDNA or genomic DNA) encoding the two heavy chains and single human light chain with a substitution of at least one non-histidine with a histidine is inserted into a replicable vector for further cloning (amplification of the DNA) and/or for expression. Many vectors are available, and generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Each component may be selected individually or based on a host cell choice or other criteria determined experimentally. Several examples of each component are known in the art.

[00150] Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the nucleic acid sequences that encode each or all the components of the bispecific antibody. A large number of promoters recognized by a variety of potential host cells are well known. These promoters are operably linked to bispecific antibody-encoding DNA by removing the promoter from the source DNA by restriction enzyme digestion and inserting the isolated promoter sequence into the vector.

[00151] Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) may also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding the bispecific antibody components. Suitable expression vectors for various embodiments include those that provide for the transient expression in mammalian cells of DNA encoding the bispecific antibody. In general, transient expression involves the use of an expression vector that is able to replicate efficiently in a host cell, such that the host cell accumulates many copies of the expression vector and, in turn, synthesizes high levels of a desired polypeptide encoded by the expression vector. Transient expression systems, comprising a suitable expression vector and a host cell, allow for the convenient positive identification of polypeptides encoded by cloned DNAs, as well as for the rapid screening of bispecific

antibodies having desired binding specificities/affinities or the desired gel migration characteristics relative to the parental antibodies having homodimers of the first or second human heavy chain variable domains.

[00152] In various embodiments, once the DNA encoding the components of the bispecific antibody are assembled into the desired vector(s) as described above, they are introduced into a suitable host cell for expression and recovery. Transfecting host cells can be accomplished using standard techniques known in the art appropriate to the host cell selected (*e.g.*, electroporation, nuclear microinjection, bacterial protoplast fusion with intact cells, or polycations, *e.g.*, polybrene, polyornithine, *etc.*).

[00153] A host cell is chosen, in various embodiments, that best suits the expression vector containing the components and allows for the most efficient and favorable production of the bispecific antibody species. Exemplary host cells for expression include those of prokaryotes and eukaryotes (single-cell or multiple-cell), bacterial cells (*e.g.*, strains of E. coli, *Bacillus spp.*, *Streptomyces spp.*, *etc.*), mycobacteria cells, fungal cells, yeast cells (*e.g.*, *S. cerevisiae*, *S. pombe*, *P. pastoris*, *P. methanolica*, *etc.*), plant cells, insect cells (*e.g.*, SF-9, SF-21, baculovirus-infected insect cells, *Trichoplusia ni*, *etc.*), non-human animal cells, human cells, or cell fusions such as, for example, hybridomas or quadromas. In various embodiments, the cell is a human, monkey, ape, hamster, rat, or mouse cell. In various embodiments, the cell is eukaryotic cell selected from CHO (*e.g.*, CHO K1, DXB-11 CHO, Veggie-CHO), COS (*e.g.*, COS-7), retinal cell, Vero, CV1, kidney (*e.g.*, HEK293, 293 EBNA, MSR 293, MDCK, HaK, BHK), HeLa, HepG2, WI38, MRC 5, Colo205, HB 8065, HL-60, (*e.g.*, BHK21), Jurkat, Daudi, A431 (epidermal), CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT 060562, Sertoli cell, BRL 3A cell, HT1080 cell, myeloma cell, tumor cell, and a cell line derived from an aforementioned cell. In various embodiments, the cell comprises one or more viral genes, *e.g.* a retinal cell that expresses a viral gene (*e.g.*, a PER.C6™ cell).

[00154] Mammalian host cells used to produce the bispecific antibody may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. Media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN^{TM}), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other supplements may also be included at appropriate

concentrations as known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are, in various embodiments, those previously used with the host cell selected for expression, and will be apparent to those skilled in the art.

[00155] The bispecific antibody may be recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysate when directly produced without a secretory signal. If the bispecific antibody is membrane-bound, it can be released from the membrane using a suitable detergent solution (*e.g.*, Triton-X 100).

[00156] Following isolation, a bispecific antibody comprising a two human heavy chains and a single human light chain derived from a rearranged human light chain variable region gene sequence selected from Vκ1-39Jκ5 and Vκ3-20Jκ1 sequences that comprise a substitution of at least one non-histidine codon with a histidine codon, is screened for its ability to exhibit pH dependent binding to one, preferably both of its antigens. The ability of bispecific antibodies to bind its antigens differently at neutral and acidic pH's (e.g., their ability to demonstrate decreased $t_{1/2}$ at acidic pH compared to neutral pH) can be determined by a variety of techniques available in the art and described in the following examples, e.g., BIACORE™ assay.

Additional Methods for Generating Antigen-Binding Proteins with pH-Dependent Antigen Binding

[00157] Various methods of generating antigen-binding proteins with pH-dependent antigen binding properties in genetically modified non-human animals described herein are provided. Also provided are methods of generating antigen binding proteins with pH-dependent antigen binding properties *in vitro*. Such methods may involve generating various components of the antigen-binding proteins *in vivo* in genetically modified non-human animals, and then modifying them and reassembling them *in vitro* outside an organism as protein complexes expressed in mammalian cell culture.

[00158] In one embodiment, the method of generating antigen-binding proteins with pH-dependent antigen binding properties utilizes an antigen-binding protein sequence, e.g., an antibody sequence, that is generated in a mouse comprising a limited repertoire of light chain variable region V and J segments, e.g., human light chain variable region V and J segments, "universal light chain" or "common light chain" mouse ("ULC" mouse), such as the mouse described in U.S. Application Publication Nos. 2011/0195454, 2012/0021409, 2012/0192300 and 2013/0045492, all incorporated herein by reference. In one embodiment, the method of generating antigen-binding proteins with pH-dependent antigen binding properties utilizes an antigen binding protein sequence that is generated in a mouse comprising a single rearranged human light chain variable region gene sequence. In one

embodiment, the method utilizes an antigen binding protein generated in a mouse comprising a single rearranged human light chain variable region gene sequence selected from human Vκ1-39Jκ5 and human Vκ3-20Jκ1.

[00159] In one embodiment, the method for generating an antigen-binding protein, e.g., an antibody, with pH dependent antigen binding properties comprises selecting a first antibody that binds to an antigen of interest (e.g., binds to an antigen of interest with a desired affinity), modifying an immunoglobulin light chain nucleotide sequence of the first antibody to comprise a substitution of at least one non-histidine codon with a histidine codon, expressing an immunoglobulin heavy chain of the first antibody and the modified immunoglobulin light chain in a cell, and selecting a second antibody expressed in the cell that retains binding to the antigen of interest (e.g., retains desired affinity for the antigen of interest) at neutral pH and displays reduced binding to the antigen of interest at an acidic pH.

[00160] In one embodiment, the method for generating an antigen-binding protein, e.g., an antibody, with pH dependent antigen binding properties comprises selecting an immunoglobulin heavy chain from an antibody (e.g., obtained from a non-human animal, e.g., a mouse, e.g., a ULC mouse) that comprises an immunoglobulin light chain having a single rearranged human immunoglobulin light chain variable region sequence wherein the antibody binds to an antigen of interest (e.g., binds to an antigen of interest with a desired affinity); modifying the nucleic acid sequence of the immunoglobulin light chain such that the single rearranged human immunoglobulin light chain variable region sequence comprises a substitution of at least one non-histidine codon with a histidine codon; expressing the selected immunoglobulin heavy chain and the immunoglobulin light chain comprising the substitution of at least one amino acid with a histidine in its variable domain; and selecting an antibody that retains binding to the antigen of interest at a neutral pH (e.g., retains desired affinity to the antigen of interest) while displaying reduced binding to the antigen of interest at an acidic pH. In various embodiments, the immunoglobulin heavy chain is derived from a rearrangement of human heavy chain variable gene segments (human V, D, and J segments).

[00161] In one embodiment, the method for generating an antigen-binding protein, e.g., an antibody, with pH-dependent antigen binding properties comprises (1) immunizing a non-human animal, e.g., a mouse, comprising a single rearranged human light chain variable region gene sequence and a repertoire of unrearranged human heavy chain variable gene segments (V, D, and J segments) with an antigen of interest and allowing a mouse to mount an immune response to said antigen, (2) selecting in the non-human animal, e.g., in the mouse, an antibody that binds to the antigen of interest with a desired affinity, (3) isolating

from the non-human animal, e.g., from the mouse, a nucleotide sequence of an immunoglobulin heavy chain of the antibody that binds to the antigen of interest with a desired affinity, (4) determining the nucleotide sequence of said heavy chain, (5) modifying a nucleotide sequence of an immunoglobulin light chain containing the single rearranged human immunoglobulin light chain variable region to comprise a substitution of at least one non-histidine codon with a histidine codon, (6) expressing the immunoglobulin heavy chain of the antibody that binds to the antigen of interest with desired affinity and the immunoglobulin light chain comprising the histidine modification in a cell, and (7) determining whether the antibody expressed in the cell retains binding to the antigen at a neutral pH while displaying reduced binding at an acidic pH. In one embodiment, the antibody expressed in the cell exhibits desired affinity to the antigen at neutral pH. In various embodiments, the immunoglobulin heavy chain is derived from a rearrangement of human heavy chain variable gene segments (human V, D, and J segments).

[00162] In one embodiment, the mouse comprising a single rearranged human light chain variable region gene sequence is a universal light chain or common light chain "ULC" mouse described in, e.g., U.S. Application Publication Nos. 2011/0195454, 2012/0021409, 2012/0192300 and 2013/0045492. In one embodiment, the single rearranged human light chain variable region gene sequence is selected from human Vκ1-39Jκ5 and human Vκ3-20Jκ1 sequence.

[00163] In one embodiment, the antigen of interest is selected from a soluble antigen, a cell surface antigen (*e.g.*, a tumor antigen) and a cell surface receptor. In a specific embodiment, the cell surface receptor is an immunoglobulin receptor. In a specific embodiment, the immunoglobulin receptor is an Fc receptor.

[00164] In one embodiment, the desired affinity of an antibody for an antigen expressed as a dissociation constant (K_D) at a neutral pH is less than 10^{-6} M, e.g., less than 10^{-8} M, e.g., less than 10^{-9} M, e.g., less than 10^{-10} M, e.g., less than 10^{-11} M, e.g., less than 10^{-12} M.

[00165] As explained above, the ULC mice, in one embodiment, comprise a single rearranged human immunoglobulin light chain variable gene sequence, and express antibodies in response to the antigen where the affinity of antibodies to the antigen is primarily mediated through the heavy chains of their antibodies. These mice comprise a repertoire of human heavy chain variable (V, D, and J) segments, that rearrange to encode a human heavy chain variable domain of an antibody that also comprises the light chain derived from the single rearranged human light chain variable sequence. In one embodiment, upon antigen exposure, these mice utilize the diverse repertoire of human heavy chain variable (V, D, and J) segments to generate an antibody with affinity to and

specificity for the antigen. Thus, upon exposure to the antigen, the nucleotide sequence of an immunoglobulin heavy chain of the antibody generated in the ULC mice may be isolated and utilized to generate a desired binding protein also comprising an immunoglobulin light chain derived from the single rearranged human immunoglobulin light chain variable region sequence (e.g., the single rearranged human immunoglobulin light chain variable region sequence with a substitution of at least one non-histidine codon with a histidine codon).

[00166] In one embodiment of the ULC mice, 90-100% of unrearranged non-human V_H gene segments are replaced with at least one unrearranged human V_H gene segment. In a specific embodiment, all or substantially all (e.g., 90-100%) of the endogenous non-human V_H gene segments are replaced with at least one unrearranged human V_H gene segment. In one embodiment, the replacement is with at least 19, at least 39, or at least 80 or 81 unrearranged human V_H gene segments. In one embodiment, the replacement is with at least 12 functional unrearranged human V_H gene segments, at least 25 functional unrearranged human V_H gene segments, or at least 43 functional unrearranged human V_H gene segments. In one embodiment, the non-human animal comprises a replacement of all non-human D_H and J_H segments with at least one unrearranged human D_H segment and at least one unrearranged human J_H segment. In one embodiment, the non-human animal comprises a replacement of all non-human D_H and J_H segments with all unrearranged human D_H segments and all unrearranged human J_H segments. Thus, the ULC mouse utilizes a diverse repertoire of human variable region gene segments (V, D, and J segments) to generate an antibody in response to the antigen of interest.

[00167] Once the heavy chain of the antibody that binds to the antigen of interest with the desired affinity is determined, the nucleotide sequence of the heavy chain is isolated and sequenced. The sequence is cloned into a vector for expression in suitable host cells, e.g., eukaryotic cells, e.g., CHO cells. In one embodiment, the sequence of a human heavy chain constant region is cloned downstream of the human heavy chain variable region sequence isolated from the mouse (e.g., from the ULC mouse).

[00168] In one embodiment, the method of generating an antigen-binding protein with pH-dependent antigen-binding properties comprises modifying a nucleotide sequence of the immunoglobulin light chain, particularly the sequence of the single rearranged human immunoglobulin light chain variable region, to comprise a substitution of at least one non-histidine codon with a histidine codon. Various techniques for modifying a nucleotide sequence are known in the art, e.g., site directed mutagenesis. In addition, a nucleotide sequence comprising the desired histidine substitution may be synthesized de novo.

[00169] In one embodiment, the substitution of at least one non-histidine codon with a histidine codon comprises a substitution resulting in expression of one, two, three, four, or more histidine residues. In one embodiment, the substitution(s) results in expression of three or four histidine residues. In one embodiment, the substitution(s) is in the immunoglobulin light chain variable region. In one embodiment, the substitution(s) is in the CDR codon, e.g., CDR1, CDR3, and/or CDR3 codon. In one embodiment, the substitution(s) is in the CDR3 codon.

[00170] In one embodiment, wherein the immunoglobulin light chain nucleic acid sequence comprises Vκ1-39Jκ5 gene sequence, and the substitution(s) is in the CDR3 codon, the substitution results in expression of a histidine at position selected from 105, 106, 108, 111, and combinations thereof. In one embodiment, the substitutions result in expression of histidines at positions 105, 106, 108, and 111. In one embodiment, the substitutions result in expression of histidines at positions 105 and 106. In one embodiment, the substitutions result in expression of histidines at positions 105 and 108. In one embodiment, the substitutions result in expression of histidines at positions 105 and 111. In one embodiment, the substitutions result in expression of histidines at positions 106 and 108. In one embodiment, the substitutions result in expression of histidines at positions 106 and 111. In one embodiment, the substitutions result in expression of histidines at positions 108 and 111. In one embodiment, the substitutions result in expression of histidines at positions 105, 106, and 108. In one embodiment, the substitutions result in expression of histidines at positions 105, 106, and 111. In one embodiment, the substitutions result in expression of histidines at positions 105, 108, and 111. In one embodiment, the substitutions result in expression of histidines at positions 106, 108, and 111. Amino acid and nucleic acid sequences of Vκ1-39Jκ5 CDR3 regions comprising various histidine substitutions are depicted in FIG. 2 and included in the sequence listing.

[00171] In one embodiment, wherein the immunoglobulin light chain nucleic acid sequence comprises Vκ3-20Jκ1 gene sequence, and the substitution(s) is in the CDR3 codon, the substitution results in expression of a histidine at position selected from 105, 106, 107, 109, and combinations thereof. In one embodiment, the substitutions result in expression of histidines at positions 105, 106, 107, and 109. In one embodiment, the substitutions result in expression of histidines at positions 105 and 106. In one embodiment, the substitutions result in expression of histidines at positions 105 and 107. In one embodiment, the substitutions result in expression of histidines at positions 105 and 109. In one embodiment, the substitutions result in expression of histidines at positions 106 and 107. In one embodiment, the substitutions result in expression of histidines at positions 106 and 109. In one embodiment, the substitutions result in expression of histidines at positions

107 and 109. In one embodiment, the substitutions result in expression of histidines at positions 105, 106, and 107. In one embodiment, the substitutions result in expression of histidines at positions 105, 106, and 109. In one embodiment, the substitutions result in expression of histidines at positions 105, 107, and 109. In one embodiment, the substitutions result in expression of histidines at positions 106, 107, and 109. Selected amino acid and nucleic acid sequences of Vκ3-20Jκ1 CDR3 regions comprising various histidine substitutions are depicted in FIG. 12 and included in the sequence listing.

[00172] Once the sequence of immunoglobulin light chain, e.g., human immunoglobulin light chain variable domain, is modified to include histidine residues at desired positions, the nucleotide sequence of the light chain is cloned into a vector for expression in suitable host cells, e.g., eukaryotic cells, e.g., CHO cells. In one embodiment, the sequence of a human light chain constant region is cloned downstream of the modified nucleotide sequence of human variable region.

[00173] In one embodiment, vectors comprising nucleotide sequence encoding modified human immunoglobulin light chain and selected human immunoglobulin heavy chain are co-expressed in a suitable host cell, e.g., eukaryotic host cell, e.g., CHO cell, to generate an antigen-binding protein. Various host cells that can be used for expression are known in the art and are mentioned throughout this specification.

[00174] An antigen-binding protein, e.g., an antibody, generated in the host cell may be secreted into cell supernatant, which is screened for proper expression and affinity for the original antigen at neutral pH. The antigen-binding protein may also be recovered from cell lysate, or, if membrane bound, released from the membrane using a suitable detergent (e.g., Triton-X). The antigen-binding protein with desired characteristics may be purified.

[00175] In one embodiment, the antigen-binding protein comprising histidine modification(s) retains the affinity to the antigen that is comparable to the affinity to the antigen of the same (original) antigen-binding protein that does not comprise histidine modification(s). In one embodiment, the affinity of the histidine-modified antigen-binding protein for the antigen of interest expressed as a dissociation constant (K_D) at a neutral pH is less than 10^{-6} M, e.g., less than 10^{-8} M, e.g., less than 10^{-9} M, e.g., less than 10^{-10} M, e.g., less than 10^{-11} M, e.g., less than 10^{-12} M.

[00176] In one embodiment, the antigen-binding protein, e.g., an antibody, comprising histidine modifications described herein exhibits pH dependent antigen binding properties. In one embodiment, the antigen-binding protein comprising histidine modifications possesses enhanced pH dependent properties over an equivalent antigen-binding protein without the histidine modifications (antigen-binding protein of the same amino acid sequence

but for the histidine modifications). In one embodiment, the antigen-binding protein described herein retains binding to the antigen at neutral pH (e.g., retains desired affinity for the antigen at neutral pH) while displaying reduced binding at an acidic pH. In one embodiment, the antigen-binding protein, e.g., the antibody, described herein, exhibits no binding to the antigen in acidic pH, while retaining binding to the antigen at neutral pH. In one embodiment, an antigen-binding protein described herein, has a decrease in dissociative half-life ($t_{1/2}$) at an acidic pH as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH of 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. In one embodiment, an antigen-binding protein described herein has a $t_{1/2}$ at an acidic pH and 37°C of about 2 min or less. In one embodiment, an antigen-binding protein described herein has a $t_{1/2}$ at an acidic pH and 37°C of less than about 1 min. In one embodiment, an antigen-binding protein described herein has a $t_{1/2}$ at an acidic pH and 25°C of about 2 min or less. In one embodiment, an antigen-binding protein described herein has a $t_{1/2}$ at an acidic pH and 25°C of less than about 1 min.

[00177] In one embodiment, the antigen-binding protein e.g., the antibody, comprising histidine modifications described herein, exhibits increased serum half life upon administration of a therapeutic dose to a subject as compared to a serum half life upon administration of an equivalent therapeutic dose of antigen-binding protein that does not comprise histidine modifications (e.g., the original antigen-binding protein that does not comprise histidine modifications). In some embodiments, the increase in serum half life upon administration of a dose of the antigen-binding protein comprising histidine modifications described herein over a serum half life upon administration of the same dose of the antigen-binding protein not comprising histidine modifications is about 2 fold, e.g., about 5 fold, e.g., about 10 fold, e.g., about 15 fold, e.g., about 20 fold, or greater. In one embodiment, serum half-life is at least about 1 day, e.g., at least about 2 days, e.g., at least about 7 days, e.g., at least about 14 days, e.g., at least about 30 days, e.g., at least about 60 days.

[00178] In addition to the *in vitro* methods for generating antigen-binding proteins with pH-dependent antigen binding properties described above, also provided herein are antigen-binding proteins, e.g., antibodies, generated by said method. In addition, said method may be utilized to generate multi-specific, e.g., bispecific, antigen-binding proteins, by selecting two different human immunoglobulin heavy chains that bind to a common (universal) light chain in a mouse, determining nucleotide sequences of the heavy chains, modifying universal light chain to comprise histidine substitutions as described above, and co-

expressing two human heavy chains with a single histidine-modified universal light chain in a host cell. Various steps for generating an antigen-binding protein described above may be applicable to the method of generating a bispecific antigen-binding protein. Bispecific antigen binding protein, confirmed to possess desired affinity for the antigen(s) and pH-dependent antigen binding properties may be purified. Thus, bispecific antibodies comprising two human heavy chains and a single human light chain comprising a human light chain variable domain sequence encoded by a human variable region gene, e.g., Vκ1-39Jκ5 or Vκ3-20Jκ1 variable region gene comprising a substitution of at least one non-histidine codon with a histidine codon, is provided.

[00179] Also provided are constructs utilized in making an antigen-binding protein comprising human immunoglobulin heavy chain and human immunoglobulin light chain comprising histidine substitutions. Host cells expressing antigen-binding proteins, e.g., antibodies, described herein are also provided.

EXAMPLES

[00180] The following examples are provided so as to describe to those of ordinary skill in the art how to make and use methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. 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. The Examples do not include detailed descriptions of conventional methods that would be well known to those of ordinary skill in the art (molecular cloning techniques, etc.). Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is indicated in Celsius, and pressure is at or near atmospheric.

Example 1. Identification of histidine residues in antigen-specific human light chains

[00181] Generation of a common light chain mouse (e.g., Vκ1-39 or Vκ3-20 common light chain mouse) and antigen-specific antibodies in those mice is described in U.S. Patent Application Nos. 13/022,759, 13/093,156, and 13/412,936 (Publication Nos. 2011/0195454, 2012/0021409, and 2012/0192300, respectively), incorporated by reference herein in their entireties. Briefly, rearranged human germline light chain targeting vector was made using VELOCIGENE® technology (see, *e.g.,* US Pat. No. 6,586,251 and Valenzuela *et al.* (2003) High-throughput engineering of the mouse genome coupled with high-resolution expression analysis, Nature Biotech. 21(6): 652-659) to modify mouse genomic Bacterial Artificial Chromosome (BAC) clones, and genomic constructs were engineered to contain a single rearranged human germline light chain region and inserted into an endogenous κ light chain locus that was previously modified to delete the endogenous κ variable and joining gene

segments. Targeted BAC DNA was then used to electroporate mouse ES cells to create modified ES cells for generating chimeric mice that express a rearranged human germline Vκ1-39Jκ5 or Vκ3-20Jκ1 region. Targeted ES cells were used as donor ES cells and introduced into an 8-cell stage mouse embryo by the VELOCIMOUSE® method (see, *e.g.,* US Pat. No. 7,294,754 and Poueymirou *et al.* (2007) F0 generation mice that are essentially fully derived from the donor gene-targeted ES cells allowing immediate phenotypic analyses Nature Biotech. 25(1): 91-99). VELOCIMICE® independently bearing an engineered human germline Vκ1-39Jκ5 or Vκ3-20Jκ1 light chain region were identified by genotyping using a modification of allele assay (Valenzuela *et al., supra*) that detects the presence of the unique rearranged human germline light chain region.

[00182] Mice bearing an engineered human germline light chain locus (ULC mice) were bred with mice that contain a replacement of the endogenous mouse heavy chain variable gene locus with the human heavy chain variable gene locus (see US 6,596,541; the VELOCIMMUNE® mouse, Regeneron Pharmaceuticals, Inc.).

[00183] VELOCIMMUNE® mouse containing a single rearranged human germline light chain region is challenged with an antigen of interest and antibodies comprising a universal light chain (e.g., Vκ1-39Jκ5) are isolated and sequenced. Amino acid sequences of selected light chains (A-K) from antigen-specific human antibodies generated in a common Vκ1-39Jκ5 light chain mouse were aligned. Histidine mutations in the CDRs of human Vκ1-39-derived light chains for a selected number of antigen-specific human antibodies were identified (FIG. 1). The partial amino acid sequence of germline Vκ1-39Jκ5 variable domain is shown above the alignments and set forth in SEQ ID NO:1, the complete variable domain amino acid sequence is set forth in SEQ ID NO:80.

Example 2. Engineering and Characterization of Histidine-Substituted Human Universal Light Chain Antibodies

Example 2.1. Engineering of Histidine Residues into a Germline Human Rearranged Light Chain

[00184] Histidine residues were engineered into a rearranged human Vκ1-39Jκ5 light chain using site directed mutagenesis primers specifically designed to introduce engineered histidine residues at Q105, Q106, Y108, and P111 positions of the human Vκ1-39Jκ5 light chain. Site directed mutagenesis was performed using molecular techniques known in the art (e.g., QuikChange II XL Site Directed Mutagenesis Kit, Agilent Technologies). Locations of the engineered residues in the CDR3 are shown in FIG. 2, the nucleic acid sequences of histidine-substituted CDR3's depicted in FIG. 2 are set forth in SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32 (corresponding amino acid sequences are set forth

in SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, and 33). The nucleic acid and amino acid sequences of germline rearranged Vκ1-39Jκ5 CDR3 are set forth in SEQ ID NOs: 2 and 3, respectively.

Example 2.2. Construction and Expression of Histidine Engineered Light Chains

[00185] Human Vκ1-39-derived light chains containing germline engineered histidine residues made according to Example 2 were constructed and paired with various human heavy chains (labeled 1-5), specific for a human cell surface receptor, to analyze expression in CHO cells. The five human heavy chains specific for a human cell surface receptor that were paired with histidine-substituted Vκ1-39-derived light chains were obtained from mice that have a single rearranged human light chain (a human Vκ1-39/Jκ5 rearranged light chain; see US2011/0195454A1).

[00186] *Enzyme-linked Immunosorbent assay (ELISA):* Antibody secretion from CHO cells was detected using an Fc ELISA, for light chains with indicated histidine modifications with five different heavy chains. The light and heavy chain sequences (but for the modifications) were generated in mice that have a single rearranged human light chain (*e.g.,* a human Vκ1-39/Jκ5 rearranged light chain; see US2011/0195454A1). Capture antibody was goat anti-human IgG and detection antibody was goat anti-human (Fc gamma-specific)-HRP. The results are shown in FIG. 3. ULC + heavy: specific heavy chain and unmodified human Vκ1-39-derived light chain. As shown in FIG. 3, expression was detected in about all mutants.

[00187] *Protein Immunoblot.* Expression in supernatants of CHO cells of paired antigen-specific heavy chains with histidine engineered light chains was further analyzed by western blot. Samples were run on a 4-12% tris-glycine gel. Results using a selected heavy chain (heavy chain 3) are shown in FIG. 4. ULC refers to a rearranged human Vκ1-39-derived light chain (as described above).

Example 2.3. Determination of Binding Affinity of Histidine Engineered Light Chains

[00188] Equilibrium dissociation constants (K_D), dissociative half-lives ($t_{1/2}$), and other kinetic parameters for selected antibody supernatants were determined by SPR (Surface Plasmon Resonance) using a BIACORE™ T200 instrument (GE Healthcare). Kinetics were measured at pH 7.4 and at pH 5.75. Results are shown in FIGs. 5A – 5E.

[00189] Numerical values for the kinetic binding properties (e.g., k_a, k_d, K_D, $t_{½}$, etc.) of antibodies binding to immunogen at neutral pH (pH 7.4) and at acidic pH (pH 5.75) were obtained using a real-time surface plasmon resonance biosensor (Biacore T200.) A Biacore CM5 sensor chip was derivatized with a mouse anti-human Fc antibody to capture

antibodies from the supernatant. A single concentration (50nM) of immunogen was then injected over the antibody-captured surface at a flow rate of 30 µl/min. Antibody-antigen association was monitored for 2.5 minutes and then the dissociation of antigen from the captured antibody was monitored for 8 minutes. Kinetic association (ka) and dissociation (kd) rate constants were determined by processing and fitting the data to a 1:1 binding with a mass transport model using Biacore T200 Evaluation software version 1.0. Equilibrium dissociation constants (K_D) and dissociative half-lives ($t_{½}$) were calculated from the kinetic rate constants as: K_D (M) = k_d / k_a; and $t_{½}$ (min) = (ln2/(60*k_d).

[00190] As shown in FIG. 5, in a binding assay of antibody to a cell surface receptor, two out of five antibodies with histidine-modified common light chains (histidine modified CDR3's of Vκ1-39/Jκ5 light chains) that were paired with the antigen-specific human heavy chains, exhibited binding to the antigen (*e.g.,* to a cell surface receptor) with different affinities at pH 7.4 and pH 5.75. Antibodies with histidine modifications that retain binding at pH 7.4, but that exhibit a low binding or no detectable binding at pH 5.75, are desirable. Antibodies with histidine modification that exhibit reduced $t_{1/2}$ at pH 5.75 as compared to pH 7.4 are desirable.

[00191] Antigen binding data for three antibodies comprising histidine-modified common light chains and three antigen-specific heavy chains (labeled 2, 3, and 6) at different pHs is summarized further in FIG. 6. These antibodies exhibited significant drop in antigen binding at pH 5.75 in comparison to pH 7.4, as demonstrated, e.g., by reduction in $t_{1/2}$ or no binding detected at pH 5.75.

Example 3. Engineering and Characterization of Genetically Modified Mouse Comprising a Histidine-Substituted Vκ1-39Jκ5 Universal Light Chain

Example 3.1. Constructing of Targeting Vector for Engineering Histidine Residues in a Rearranged Human Light Chain Variable Region

[00192] A genetically modified mouse containing a rearranged human light chain gene having histidine residues engineered into a CDR region of the human light chain is made using targeting vectors made by standard molecular cloning techniques known in the art.

[00193] Briefly, various rearranged human germline light chain targeting vectors are made using VELOCIGENE® technology (see, *e.g.,* US Pat. No. 6,586,251 and Valenzuela *et al.* (2003) High-throughput engineering of the mouse genome coupled with high-resolution expression analysis, Nature Biotech. 21(6):652-659) to modify mouse genomic Bacterial Artificial Chromosome (BAC) DNA to contain a single rearranged human germline light chain region and inserted into an endogenous κ light chain locus that was previously modified to

delete the endogenous κ variable and joining gene segments. The rearranged human germline light chain region is modified at one or more nucleotide positions within the sequence of the light chain to encode histidine residues that are not normally present at the respective locations of the germline sequence. The targeting vectors are electroporated into mouse embryonic stem (ES) cells and confirmed using a quantitative PCR assay (*e.g.,* TAQMAN™).

[00194] Specifically, a strategy for constructing these targeting vectors is shown in FIGs. 8A – 8F. A plasmid used for generating a targeting vector for common (universal) light chain mouse ("ULC mouse," described in, e.g., US2011/0195454A1), containing pBS + FRT-Ub-Hyg-FRT + mouse Vκ3-7 leader +human Vκ1-39Jκ5 was modified by site directed mutagenesis (QuickChange II XL Kit) to replace Q105, Q106, Y108 and P111 or Q106, Y108 and P111 with histidine residues in the CDR3 region using site-directed mutagenesis primers shown in FIG. 7 (See FIG. 8A for this engineering step). Resultant vectors (H105/106/108/111 and H106/108/111) were modified further and ligated into a vector comprising mouse Igκ constant region, mouse enhancers, a mouse 3' homology arm and a SPEC cassette (FIG. 8B). Further modification involved ligation into a vector carrying 5' mouse arm and comprising Frt-Ub-NEO-Frt cassette (FIG. 8B). Resultant targeting vectors were electroporated into ES cells comprising deletion of the mouse Igκ variable locus (comprising κ variable and joining gene segments) (FIGs. 8C-8F).

[00195] Positive ES cell clones were confirmed by using a modification of allele assay (Valenzuela et al.) using probes specific for the engineered Vκ1-39Jκ5 light chain region inserted into the endogenous κ light chain locus. Primers and probes used in the assay are shown in Table 1 below and set forth in the Sequence Listing; the locations of the probes are depicted in FIGs. 8C-8F.

Table 1: Primers and Probes Used for ES Cell Screening

Probe Name	**Assay**	**Probe Sequence**	**5' Primer**	**3' Primer**
Neo	GOA	TGGGCACAACA GACAATCGGCTG (SEQ ID NO:38)	GGTGGAGAGG CTATTCGGC (SEQ ID NO:39)	GAACACGGCGG CATCAG (SEQ ID NO:40)
ULC-m1	GOA	CCATTATGATGC TCCATGCCTCTC	AGGTGAGGGT ACAGATAAGTG	TGACAAATGCCC TAATTATAGTGAT

Probe Name	Assay	Probe Sequence	5' Primer	3' Primer
		TGTTC (SEQ ID NO:41)	TTATGAG (SEQ ID NO:42)	CA (SEQ ID NO:43)
1633h2 (Vκ1-39Jκ5-specific)	GOA	ATCAGCAGAAAC CAGGGAAAGCCC CT (SEQ ID NO:44)	GGGCAAGTCA GAGCATTAGCA (SEQ ID NO:45)	TGCAAACTGGAT GCAGCATAG (SEQ ID NO:46)
mIgKd2	Retention	GGCCACATTCCA TGGGTTC (SEQ ID NO:47)	GCAAACAAAAA CCACTGGCC (SEQ ID NO:48)	CTGTTCCTCTAAA ACTGGACTCCAC AGTAAATGGAAA (SEQ ID NO:49)
mIgKp15	Retention	GGGCACTGGATA CGATGTATGG (SEQ ID NO:50)	CACAGCTTGTG CAGCCTCC (SEQ ID NO:51)	AGAAGAAGCCTG TACTACAGCATCC GTTTTACAGTCA (SEQ ID NO:52)

[00196] The NEO selection cassette introduced by the targeting constructs was deleted by transfecting ES cells with a plasmid that expresses FLP (FIGs. 8C and 8E). Optionally, the neomycin cassette may be removed by breeding to mice that express FLP recombinase (*e.g.,* US 6,774,279). Optionally, the neomycin cassette is retained in the mice.

[00197] Targeted ES cells 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 Pat. No. 7,294,754 and Poueymirou et al. (2007) F0 generation mice that are essentially fully derived from the donor gene-targeted ES cells allowing immediate phenotypic analyses *Nature Biotech.* 25(1):91-99. VELOCIMICE® independently bearing an engineered human light chain gene that contains histidine residues mutated into one or more positions along the sequence were made from the targeted ES cells described above.

[00198] Pups were genotyped and pups heterozygous for the engineered histidine-modified human light chain were selected for characterizing expression of the light chain and

binding capabilities of the expressed antibodies. Primers and probes for genotyping of mice specifically comprising a universal light chain gene with either three (H106/108/111; "1930") or four (H105/105/108/111; "1927") histidine modifications are listed in Table 2 below and set forth in the Sequence Listing. Mice containing histidine modification in their universal light chains are referred herein as "HULC" mice (histidine universal light chain mice).

Table 2: Primers and Probes Used for Genotyping

Probe Name	Assay	Probe Sequence	5' Primer	3' Primer
1927jxn3	GOA 1927 (4 His) mouse-specific	ACCATAGTCACAGTACCCA (SEQ ID NO:53)	AGCAGTCTGCAACCTGAAGATTT (SEQ ID NO:54)	CCCTTGGCCGAAGGTGAT (SEQ ID NO:55)
1930jxn3	GOA 1930 (3 His) mouse-specific	ATAGTCACAGTACCCATCC (SEQ ID NO:56)	AGTCTGCAACCTGAAGATTTTGC (SEQ ID NO:57)	CCCTTGGCCGAAGGTGAT (SEQ ID NO:58)

Example 3.2. Analysis of Immune Response to Antigen in Mice with Histidine-Substituted Universal Light Chains

[00199] Cell surface receptor ("Antigen A") was used as the immunogen to immunize mice that were either heterozygous for expression of a pre-arranged human kappa light chain utilizing Vk1-39 and Jk5 that has 4 histidine substitutions in CDR3 (hereinafter "HULC 1927") or heterozygous for expression of a pre-arranged human kappa light chain utilizing Vk1-39 and Jk5 that has 3 histidine substitutions in CDR3 (hereinafter "HULC1930"), or homozygous WT mice. Pre-immune serum was collected from the mice prior to the initiation of immunization. The immunogen was administered at 2.35 µg of protein for the initial priming immunization mixed with 10 µg of CpG oligonucleotide as an adjuvant (Invivogen) in a volume of 25 µl via footpad (f.p.). Subsequently, mice were boosted via the same route with 2.35 µg of Antigen A along with 10 µg of CpG and 25 µg of Adju-Phos (Brenntag) as adjuvants on days 3, 6, 11, 13, 17, 20 for a total of 6 boosts. The mice were bled on days 15 and 22 after the 4th and 6th boost, respectively. Their antiserum was assayed for antibody titers to Antigen A.

[00200] Antibody serum titers against immunogen were determined by a standard ELISA. To perform the ELISA, 96-well microtiter plates (Thermo Scientific) were coated at 2 µg/ml

with Antigen A in phosphate-buffered saline (PBS, Irvine Scientific) overnight at 4ºC. The next day, plates were washed with phosphate-buffered saline containing 0.05% Tween 20 (PBS-T, Sigma-Aldrich) four times using a plate washer (Molecular Devices). Plates were then blocked with 250 µl of 0.5% bovine serum albumin (BSA, Sigma-Aldrich) in PBS and incubated for 1 hour at room temperature. The plates were then washed four times with PBS-T. Sera from immunized mice and pre-immune sera were serially diluted three-fold in 0.5% BSA-PBS starting at 1:300 or 1:1000, added to the blocked plates in duplicate, and then incubated for 1 hour at room temperature. The last two wells were left blank to be used as a secondary antibody control (background control). The plates were again washed four times with PBS-T in a plate washer. Goat anti-mouse IgG-Fc- Horse Radish Peroxidase (HRP) conjugated secondary antibody (Jackson Immunoresearch) was then added to the plates at 1:5000/1:10,000 dilution and incubated for 1 hour at room temperature. Plates were then washed eight times with PBS-T and developed using TMB/H_2O_2 as substrate. The substrate was incubated for 20 min and the reaction was stopped with 2 N sulfuric acid (H_2SO_4, VWR, cat# BDH3500-1) or 1 N phosphoric acid (JT Baker, Cat# 7664-38-2). Plates were read on a spectrophotometer (Victor, Perkin Elmer) at 450nm. Antibody titers were computed using Graphpad PRISM software.

[00201] The immune response induced in mice to the injected immunogen is represented as antibody titers, which is defined as the reciprocal of the highest serum dilution at which antigen binding absorbance is two-fold higher over background. Therefore, the higher the number, the greater the humoral immune response to the immunogen. Antibody titers induced to the immunogen were very high in both strains of HULC mice and in the WT mice, with no significant differences observed among the strains (FIG. 9).

Example 3.3. Generation of pH-Sensitive Monoclonal Antibodies

[00202] When a desired immune response to the immunogen was achieved in both strains of HULC mice and in the WT mice, splenocytes from each mouse strain were harvested and fused with mouse myeloma cells to generate hybridoma cells, which were allowed to grow in 96-well plates. After 10 days of growth, supernatants from each hybridoma cell-containing well were screened via immunogen-specific ELISA to identify positive antigen binding samples. For the ELISA, 96 well micro-titer plates were coated with 1ug/mL of an anti-myc polyclonal antibody (Novus Biologicals, #NB600-34) overnight at 4°C to immobilize the myc-tagged antigen, followed by blocking with a solution of 0.5% (w/v) BSA in PBS. The plates were washed, the antigen solutions were added to the plates at a concentration of 1 µg/mL and allowed to bind to the coated plate for 1 hour at room temperature. Subsequently, supernatants from hybridoma cells were added to the wells at 1:50 dilution and allowed to bind for 1 hour at room temperature. The plate bound

antibodies were detected using an anti-mouse IgG polyclonal antibody conjugated with HRP (Jackson Immunoresearch, #115-035-164). TMB substrates were added to the plates (BD Biosciences, # 51-2606KC/51-2607KC) and colorimetric signals were developed according to manufacturer recommended protocol. The absorbance was recorded at 450nm on a Victor Wallac plate reader. Antigen positive samples defined as having an OD equal to or greater than 0.5 (with the baseline having OD of about 0.1) were subject to affinity screening using a real-time surface plasmon resonance biosensor (Biacore 4000).

[00203] Kinetic binding parameters (e.g., k_a, k_d, K_D, $t_{½}$, etc.) for antibody binding to the immunogen at neutral pH (pH 7.4) and at acidic pH (pH 6.0) were recorded. A Biacore CM4 sensor chip was derivatized with a polyclonal goat anti-mouse Fc antibody to capture antibodies from the supernatant. A single concentration (100nM) of immunogen was then injected over the antibody-captured surface at a flow rate of 30 µl/min. Antibody-antigen association was monitored for 1.5 minutes and then the dissociation of antigen from the captured antibody was monitored for 2.5 minutes. Kinetic association (k_a) and dissociation (k_d) rate constants were determined by processing and fitting the data to a 1:1 binding with a mass transport model using Biacore 4000 Evaluation software version 1.0. Equilibrium dissociation constants (K_D) and dissociative half-lives ($t_{½}$) were calculated from the kinetic rate constants as: K_D (M) = k_d / k_a; and $t_{½}$ (min) = ln2/(60*k_d). A set of samples that displayed decreased binding at pH 6.0 as compared to that at pH 7.4 (pH sensitive) as well as a set of control samples that displayed no significant rate changes between the pH 7.4 and pH 6.0 (pH insensitive controls) were selected to be produced clonally. FIG. 10 depicts comparison of the number of total antigen positives and the number of antigen positives displaying pH-sensitive antigen binding from HULC and WT mice.

[00204] Among the antigen positives, 18 and 7 clones isolated from two heterozygous HULC1927 mice and two HULC1930 respectively, and 1 clone from the WT mouse, were made monoclonal. Supernatants of the monoclonal hybridomas were subject to neutral and low pH antigen dissociation rate (off-rate) analysis and cell pellets were used for light chain variable domain DNA sequencing.

Example 3.4. Sequencing and Somatic Hypermutations in CDR3 Region of Vκ1-39Jκ5-based Histidine Universal Light Chain Mice

[00205] Cell pellets from monoclonal hybridomas from HULC and WT mice were used for light chain variable domain DNA sequencing. From the 26 clones made monoclonal (see Example 3.3 above) and subjected to sequencing, 15 were confirmed as using either a HULC or WT mouse light chain (MM and NN, see Table 4). 14 clones were derived from

HULC heterozygous mice (1927 or 1930 mice) and 1 was derived from a WT mouse (OO, see Table 4).

[00206] From the 14 antigen positive samples derived from HULC heterozygous mice, 12 of the monoclonal antibodies utilized their corresponding HULC light chain, while 2 utilized a WT mouse light chain. All but one of the HULC utilizing antibodies retained all of the introduced histidine mutations as shown in Table 3 (italicized antibody). Sequencing of clone AA produced 2 different HULC sequences, which is reflected by two entries in Table 3.

Table 3: Number of conserved histidine insertions and somatic hypermutations in light chain sequences from clones utilizing the HULC light chain

		Light Chain Sequences from mice utilizing HULC		
Clone Name	**Mouse Strain**	**# Conserved His Mutations in CDR3**	**# Somatic Hypermutations in Framework**	**# Somatic Hypermutations in CDRs**
AA (Sequence 1)	1927	4	3	0
AA (Sequence 2)	1927	4	1	1
BB	1927	4	3	3
CC	1927	4	0	0
DD	*1927*	*3*	*1*	*1*
EE	1927	4	2	2
FF	1927	4	0	1
GG	1927	4	1	1
HH	1927	4	2	0
II	1930	3	1	1
JJ	1930	3	4	5
KK	1930	3	1	2
LL	1930	3	1	0

Example 3.5. pH-Dependent Binding of Monoclonal Antibodies Generated in Vκ1-39Jκ5-based Histidine Universal Light Chain Mice

[00207] In order to further assess the pH-dependent binding characteristics of the monoclonal antibodies isolated from HULC and WT mice, binding experiments were carried out in which the antibody/antigen association phase was observed at neutral pH and the antibody/antigen dissociation phase was observed at either neutral or acidic pHs.

[00208] A Biacore CM4 sensor chip was derivatized with a polyclonal rabbit anti-mouse Fc antibody. Monoclonal antibody supernatants were captured onto the anti-mouse Fc sensor surface. Two concentrations, 50 nM (in duplicate) and 16.7 nM, of the immunogen

were injected over the monoclonal antibody captured surface at a flow rate of 30 µl/min. Antibody-antigen association was monitored at pH 7.4 for 4 minutes and then the dissociation of antigen from the captured monoclonal antibody was monitored for 15 minutes at either pH 7.4 or 6.0. Dissociation (k_d) rate constants were determined by processing and fitting the data using Scrubber version 2.0 curve fitting software and are shown in Table 4. Dissociative half-lives ($t_{1/2}$) were calculated from the dissociation rate constants as: $t_{1/2}$ (min) = (ln2/k_d)/60, and are shown in Table 4. Sensorgrams depicting the association/dissociation characteristics of several antibodies listed in Table 4 under the various pH conditions are shown graphically in FIG 11. The individual lines in each graph represent the binding responses at different concentrations of the respective antibodies. All experiments were carried out at 25ºC. Dissociative half-life values (t½) are noted above the respective sensorgrams. Response is measured in RU.

Table 4. Dissociation (k_d) rate constants and dissociative half-lives ($t_{1/2}$) of monoclonal HULC or WT antibodies binding to their immunogen at neutral and low pH.

		pH 7.4 Association/pH 7.4 Dissociation				**pH 7.4 Association/pH 6.0 Dissociation**				**pH 6.0/pH7.4 ratio**	
Clone Name	**Light chain used**	**neutral mAb cap-ture**	**50nM immuno-gen bound (RU)**	**k_d (1/s)**	**t½ (min)**	**low mab cap-ture**	**50nM immuno-gen bound (RU)**	**k_d (1/s)**	**t½ (min)**	**k_d**	**t½**
AA	HULC (1927)	129	70	5.60E-05	206	122	73	2.18E-04	53	3.9	0.3
BB	HULC (1927)	350	165	6.00E-04	19	378	185	2.20E-03	5	3.7	0.3
CC	HULC (1927)	611	251	2.03E-04	57	545	226	6.68E-03	2	33.0	0.03
DD	HULC (1927)	182	75	3.55E-04	33	168	74	6.44E-04	18	1.8	0.6
HH	HULC (1927)	268	92	1.36E-04	85	251	91	5.39E-04	21	4.0	0.3
GG	HULC (1927)	353	110	2.78E-04	42	328	102	8.97E-04	13	3.2	0.3
FF	HULC (1927)	334	202	4.79E-05	241	364	220	6.90E-05	167	1.4	0.7
EE	HULC (1927)	339	124	5.08E-04	23	299	120	4.66E-04	25	0.9	1.1
II	HULC (1930)	387	174	1.22E-04	95	334	147	2.14E-04	54	1.8	0.6
JJ	HULC (1930)	363	14	9.83E-04	12	333	12	5.30E-04	22	0.5	1.9

		pH 7.4 Association/pH 7.4 Dissociation				pH 7.4 Association/pH 6.0 Dissociation				pH 6.0/pH7.4 ratio	
Clone Name	**Light chain used**	**neutral mAb capture**	**50nM immunogen bound (RU)**	**k_d (1/s)**	**t½ (min)**	**low mab capture**	**50nM immunogen bound (RU)**	**k_d (1/s)**	**t½ (min)**	**k_d**	**t½**
KK	HULC (1930)	490	303	7.41E-05	156	484	295	1.29E-04	90	1.7	0.6
LL	HULC (1930)	636	41	3.09E-04	37	597	36	5.77E-04	20	1.9	0.5
MM*	WT (from 1927 mouse)	245	6	NA	NA	203	6	NA	NA	NA	NA
NN	WT (from 1927 mouse)	394	231	5.26E-04	22	378	231	9.35E-04	12	1.8	0.6
OO	WT	413	89	2.94E-04	39	400	83	3.57E-04	32	1.2	0.8

*k_d and $t_{1/2}$ values could not be determined due to low antigen binding signal

Example 4. Engineering of Genetically Modified Mouse Comprising a Histidine-Substituted Vκ3-20Jκ1 Universal Light Chain

[00209] A mouse comprising a common Vκ3-20Jκ1 light chain was generated as described in, e.g., U.S. Patent Application Nos. 13/022,759, 13/093,156, 13/412,936, and 13/488,628 (Publication Nos. 2011/0195454, 2012/0021409, 2012/0192300, and 2013/0045492, respectively), and in Example 1 above. The amino acid sequence of the germline universal Vκ3-20Jκ1 light chain variable domain is set forth in SEQ ID NO:59.

[00210] Histidine substitutions were introduced into the Vκ3-20Jκ1 universal light chain targeting vector and mice generated from the same using a similar strategy to the one described above in Example 3 for Vκ1-39Jκ5 histidine modified universal light chain mice (HULC 1927 and 1930).

[00211] Briefly, the strategy for generating a histidine-modified Vκ3-20Jκ1 universal light chain targeting vector is summarized in FIGs. 14A-14D. A plasmid used for generating a targeting vector for common (universal) light chain mouse ("ULC mouse," described in, e.g., US2011/0195454A1), containing pBS + FRT-Ub-Hyg-FRT + mouse Vκ3-7 leader +human Vκ3-20Jκ1 was modified by site directed mutagenesis (QuickChange Lightning Kit) to replace Q105, Q106, Y107 and S109 or Q105, Q106 and S109 (see alignment in FIG. 12) with histidine residues in the CDR3 region using site-directed mutagenesis primers shown in

FIG. 13 (See FIG. 14A for this engineering step). Resultant vectors (H105/106/107/109 and H105/106/109) were modified further and ligated into a vector comprising mouse Igκ constant region, mouse enhancers, a mouse 3' homology arm and a SPEC cassette (FIG. 14B). Further modification involved ligation into a vector carrying 5' mouse arm and comprising Frt-UB-NEO-Frt cassette (FIG. 14B). Resultant targeting vectors were electroporated into ES cells comprising deletion of the mouse Igκ variable locus (comprising κ variable and joining gene segments) (FIGs. 14C-14D).

[00212] Positive ES cell clones were confirmed by using a modification of allele assay (Valenzuela et al.) using probes specific for the engineered Vκ3-20κJ1 light chain region inserted into the endogenous κ light chain locus. Primers and probes used in the assay are shown in Table 5 below and set forth in the Sequence Listing; the locations of the probes are depicted in FIGs. 14C-14D.

Table 5: Primers and Probes Used for ES Cell Screening

Probe Name	**Assay**	**Probe Sequence**	**5' Primer**	**3' Primer**
Neo	GOA	TGGGCACAACA GACAATCGGCTG (SEQ ID NO:38)	GGTGGAGAGG CTATTCGGC (SEQ ID NO:39)	GAACACGGCGG CATCAG (SEQ ID NO:40)
ULC-m1	GOA	CCATTATGATGCT CCATGCCTCTCT GTTC (SEQ ID NO:41)	AGGTGAGGGT ACAGATAAGTG TTATGAG (SEQ ID NO:42)	TGACAAATGCCC TAATTATAGTGAT CA (SEQ ID NO:43)
1635h2 (Vκ3-20Jκ1 specific)	GOA	AAAGAGCCACCC TCTCCTGCAGGG (SEQ ID NO:65)	TCCAGGCACCC TGTCTTTG (SEQ ID NO:66)	AAGTAGCTGCTG CTAACACTCTGAC T (SEQ ID NO:67)
mIgKd2	Retention	GGCCACATTCCA TGGGTTC (SEQ ID NO:47)	GCAAACAAAAA CCACTGGCC (SEQ ID NO:48)	CTGTTCCTCTAAA ACTGGACTCCAC AGTAAATGGAAA

Probe Name	Assay	Probe Sequence	5' Primer	3' Primer
				(SEQ ID NO:49)
mIgKp15	Retention	GGGCACTGGATA CGATGTATGG (SEQ ID NO:50)	CACAGCTTGTG CAGCCTCC (SEQ ID NO:51)	AGAAGAAGCCTG TACTACAGCATCC GTTTTACAGTCA (SEQ ID NO:52)

[00213] The NEO selection cassette introduced by the targeting constructs is deleted by transfecting ES cells with a plasmid that expresses FLP (FIGs. 14C and 14D). Optionally, the neomycin cassette may be removed by breeding to mice that express FLP recombinase (*e.g.,* US 6,774,279). Optionally, the neomycin cassette is retained in the mice.

[00214] Targeted ES cells described above are used as donor ES cells and introduced into an 8-cell stage mouse embryo by the VELOCIMOUSE® method (see, *e.g.,* US Pat. No. 7,294,754 and Poueymirou et al. (2007) F0 generation mice that are essentially fully derived from the donor gene-targeted ES cells allowing immediate phenotypic analyses *Nature Biotech.* 25(1):91-99). VELOCIMICE® independently bearing an engineered human light chain gene that contains histidine residues mutated into one or more positions along the sequence are made from the targeted ES cells described above.

[00215] Pups are genotyped and pups heterozygous for the engineered histidine-modified human light chain are selected for characterizing expression of the light chain and binding capabilities of the expressed antibodies. Primers and probes for genotyping of mice specifically comprising a universal light chain gene with either three (H105/106/109; “6183”) or four (H105/105/108/111; “6181”) histidine modifications are listed in Table 6 below and set forth in the Sequence Listing. Mice containing histidine modification in their universal light chains are referred herein as “HULC” mice (histidine universal light chain mice).

Table 6: Primers and Probes Used for Genotyping

Probe Name	Assay	Probe Sequence	5' Primer	3' Primer
hVI494-1	GOA 6181 (4 His) mouse-	CTGTCATCACCATG G	GCAGACTGGAGC CTGAAGATTTT	CCGAACGTCCAAGG TGAGTG

	specific	(SEQ ID NO:68)	(SEQ ID NO:69)	(SEQ ID NO:70)
hVl495-1	GOA 6183 (3 His) mouse-specific	TACTGTCATCACTATGG (SEQ ID NO:71)	GCAGACTGGAGCCTGAAGATTT (SEQ ID NO:72)	CCGAACGTCCAAGGTGAGTG (SEQ ID NO:73)

[00216] Mice are immunized with antigen of interest and tested for ability to generate antibodies with pH-dependent binding.

Example 5. Breeding of Mice Comprising a Histidine-Substituted Single Rearranged Human Universal Light Chain Mouse (HULC)

[00217] This Example describes several other genetically modified mouse strains that can be bred to any one of the HULC mice described herein to create multiple genetically modified mouse strains harboring multiple genetically modified immunoglobulin loci.

[00218] *Endogenous Igλ Knockout (KO).* To optimize the usage of the engineered light chain locus, any one of the HULC animals described above (e.g., comprising Vκ1-39Jκ5 or Vκ3-20Jκ1 histidine-substituted universal light chain) may be bred to another mouse containing a deletion in the endogenous λ light chain locus. In this manner, the progeny obtained will express, as their only light chain, the rearranged histidine-substituted human germline light chain region as described in Examples 3 and 4 above. Breeding is performed by standard techniques recognized in the art and, alternatively, by a commercial breeder (*e.g.,* The Jackson Laboratory). Mouse strains bearing an engineered histidine-substituted light chain locus and a deletion of the endogenous λ light chain locus are screened for presence of the unique light chain region and absence of endogenous mouse λ light chains.

[00219] *Humanized Endogenous Heavy Chain Locus.* Mice bearing an engineered human germline light chain locus (HULC mice) are bred with mice that contain a replacement of the endogenous mouse heavy chain variable gene locus with the human heavy chain variable gene locus (see US 6,596,541; the VELOCIMMUNE® mouse, Regeneron Pharmaceuticals, Inc.). The VELOCIMMUNE® mouse comprises a genome comprising human heavy chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces antibodies comprising a human heavy chain variable domain and a mouse heavy chain constant region in response to antigenic stimulation.

[00220] Mice bearing a replacement of the endogenous mouse heavy chain variable region locus with the human heavy chain variable region locus and a histidine-substituted single rearranged human light chain variable region at the endogenous κ light chain locus

are obtained. Reverse chimeric antibodies containing somatically mutated heavy chains (human heavy chain variable domain and mouse C_H) with a histidine-substituted single human light chain (HULC, human light chain variable domain and mouse C_L) are obtained upon immunization with an antigen of interest. pH-dependent human antibodies generated in such mice are identified using antibody isolation and screening methods known in the art or described above. Variable light and heavy chain region nucleotide sequences of B cells expressing the antibodies, e.g., pH-sensitive antibodies, are identified, and fully human antibodies are made by fusion of the variable heavy and light chain region nucleotide sequences to human C_H and C_L nucleotide sequences, respectively, in a suitable expression system.

Equivalents

[00221] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

[00222] Entire contents of all non-patent documents, patent applications and patents cited throughout this application are incorporated by reference herein in their entirety.

What is claimed is:

1. A method of generating an antibody that exhibits pH-dependent binding to an antigen of interest comprising:

(a) co-expressing in vitro in a host cell:

(i) a first immunoglobulin heavy chain nucleotide sequence comprising a heavy chain variable region sequence encoding a heavy chain variable domain of a first antibody, wherein the first antibody binds to the antigen of interest with a desired affinity at a neutral pH, wherein the heavy chain variable region sequence is generated in a non-human animal comprising in its germline genome a nucleic acid sequence comprising a single rearranged human immunoglobulin light chain variable region sequence that comprises a human germline Vκ1-39 or Vκ3-20 gene segment rearranged with a human germline Jκ gene segment and that is expressed in the antibody repertoire of the non-human animal, and

(ii) an immunoglobulin light chain nucleotide sequence that is (1) derived from the single rearranged human immunoglobulin light chain variable region sequence and (2) modified to comprise a substitution of at least one non-histidine codon with a histidine codon designed to express a histidine in the Vκ1-39 or Vκ3-20 gene segment at a position selected from 105, 106, 107, 108, 109, 111 and a combination thereof (according to IMGT numbering), and

(b) selecting a second antibody expressed in the host cell that is encoded by the first immunoglobulin heavy chain nucleotide sequence and the immunoglobulin light chain nucleotide sequence, wherein the second antibody retains the desired affinity for the antigen of interest at neutral pH and displays reduced binding to the antigen of interest at an acidic pH.

2. The method of claim 1, wherein the non-human animal further comprises an immunoglobulin heavy chain sequence derived from a repertoire of human VH, DH, and JH segments present in the non-human animal germline genome.

3. The method of claim 1, wherein the single rearranged human immunoglobulin light chain variable region sequence is Vκ1-39/Jκ5, and the substitution of at least one non-histidine codon with a histidine codon in the immunoglobulin light chain nucleotide sequence is designed to express a histidine at a position selected from 105, 106, 108, 111, and a combination thereof.

4. The method of claim 1 wherein the single rearranged human immunoglobulin light chain variable region sequence is Vκ3-20/Jκ1, and the substitution of at least one non-histidine codon with a histidine codon in the immunoglobulin light chain nucleotide sequence is designed to express a histidine at a position selected from 105, 106, 107, 109, and a combination thereof.

5. The method of claim 1, wherein the second antibody displays a decrease in dissociative half-life ($t_{1/2}$) at an acidic pH as compared to neutral pH of 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.

6. The method of claim 1, wherein the second antibody exhibits a dissociative half-life ($t_{1/2}$) at acidic pH and 37° C. of about 2 minutes or less.

7. The method of claim 1, wherein the non-human animal is a mouse.

8. The method of claim 2, wherein the non-human animal is a mouse.

9. The method of claim 1, wherein the second antibody comprises a fully human heavy chain and a fully human light chain.

10. The method of claim 1, wherein the immunoglobulin light chain nucleotide sequence of the first antibody further comprises at least one codon that encodes for a somatic mutation not encoded by the human germline Vκ1-39 or Vκ3-20 gene segment rearranged with a human germline Jκ gene segment.

11. The method of claim 10, wherein the second antibody comprises a fully human first immunoglobulin heavy chain and a fully human immunoglobulin light chain.

12. A method of generating a bi-specific antibody that exhibits pH-dependent binding to a first antigen of interest and a second antigen of interest comprising:

(a) co-expressing in vitro in a host cell:

(i) a first immunoglobulin heavy chain nucleotide sequence comprising a first heavy chain variable region sequence encoding a first heavy chain variable domain of a first antibody, wherein the first antibody binds the first antigen of interest with a desired affinity at a neutral pH,

(ii) a second immunoglobulin heavy chain nucleotide sequence comprising a second heavy chain variable region sequence encoding a second heavy chain variable domain of a second antibody, wherein the second antibody binds the second antigen of interest with a desired affinity at a neutral pH,

wherein the first and second heavy chain variable region sequences are generated in a non-human animal comprising in its germline genome a nucleic acid sequence comprising a single rearranged human immunoglobulin light chain variable region sequence that comprises a human germline Vκ1-39 or Vκ3-20 gene segment rearranged with a human germline Jκ gene segment and that is expressed in the antibody repertoire of the non-human animal and (2) comprise a same light chain component encoded by the human germline Vκ1-39 or Vκ3-20 gene segment rearranged with a human germline Jκ gene segment; and

(iii) an immunoglobulin light chain nucleotide sequence that is (1) derived from the single rearranged human immunoglobulin light chain variable region sequence and (2) modified to comprise a substitution of at least one non-histidine codon with a histidine codon designed to express a histidine in the Vκ1-39 or Vκ3-20 gene segment at a position selected from 105, 106, 107, 108, 109, 111 and a combination thereof (according to IMGT numbering), and

(b) selecting a third antibody expressed in the host cell that is encoded by the first and second immunoglobulin heavy chain nucleotide sequences and the immunoglobulin light chain nucleotide sequence, wherein the third antibody retains a desired affinity for the first and second antigens of interest at a neutral pH, and displays reduced binding to the first and second antigens of interest at an acidic pH.

13. The method of claim 12, wherein first and second antibodies are generated in a non-human animal further comprising an immunoglobulin heavy chain sequence derived from a repertoire of human VH, DH, and JH segments present in a non-human animal germline genome.

14. The method of claim 12, wherein the single rearranged human immunoglobulin light chain variable region sequence is Vκ1-39/Jκ5, and the substitution of at least one non-histidine codon with a histidine codon in the immunoglobulin light chain nucleotide sequence is designed to express a histidine at a position selected 105, 106, 108, 111, and a combination thereof.

15. The method of claim 12 wherein the single rearranged human immunoglobulin light chain variable region sequence is Vκ3-20/Jκ1, and the substitution of at least one non-histidine codon with a histidine codon in the immunoglobulin light chain nucleotide sequence is designed to express a histidine at a position selected from 105, 106, 107, 109, and a combination thereof.

16. The method of claim 12, wherein the non-human animal is a mouse.

17. The method of claim 13, wherein the non-human animal is a mouse.

18. The method of claim 12, wherein the third antibody comprises a fully human first immunoglobulin heavy chain, a fully human second immunoglobulin heavy chain, and a fully human immunoglobulin light chain.

19. The method of claim 12, wherein the third antibody displays a decrease in dissociative half-life ($t_{1/2}$) at an acidic pH as compared to neutral pH of 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.

20. The method of claim 12, wherein the third antibody exhibits a dissociative half-life ($t_{1/2}$) at acidic pH and 37° C. of about 2 minutes or less.