HUMANIZED NON-HUMAN ANIMALS WITH RESTRICTED IMMUNOGLOBULIN HEAVY CHAIN LOCI

FIELD

[0001] Genetically engineered non-human animals that comprise a reduced immunoglobulin heavy chain variable gene complexity are provided, wherein the non-human animals are capable of expressing an ADAM6 protein or functional fragment thereof. Genetically engineered non-human animals that express antibodies from a restricted number of immunoglobulin heavy chain variable gene segments and/or variants thereof, wherein the non-human animals lack a functional endogenous ADAM6 gene but retain ADAM6 function, are described, including mice that comprise a modification of an endogenous immunoglobulin heavy chain variable (V_H) region locus that renders the mouse incapable of making a functional ADAM6 protein and results in a loss in fertility. The genetically modified mice comprise an immunoglobulin V_H locus characterized by a restricted number of V_H gene segments, *e.g.*, a single immunoglobulin V_H segment, *e.g.*, a human V_H1-69 gene segment or a human V_H1-2 gene segment, and that further comprise ADAM6 function are described, including mice that comprise an ectopic nucleic acid sequence that restores fertility to a male mouse.

[0002] Genetically modified mice, cells, embryos, and tissues that comprise a nucleic acid sequence encoding a functional ADAM6 locus are described, wherein the mice, cells, embryos, and tissues express an immunoglobulin heavy chain derived from a single human V_H gene segment. Further, the mice, cells, embryos, and tissues lack a functional endogenous ADAM6 gene but retain ADAM6 function characterized by the presence of an ectopic nucleic acid sequence that encodes an ADAM6 protein. Methods for making antibody sequences in fertile non-human animals that are useful for binding pathogens, including human pathogens are provided.

BACKGROUND

[0003] Non-human animals, *e.g.*, mice, have been genetically engineered to be useful tools in methods for making antibody sequences for use in antibody-based human therapeutics. Mice with humanized variable region loci (*e.g.*, V_H, D_H, and J_H genes, and V_L and J_L genes) are used to generate cognate heavy and light chain variable domains for use in antibody therapeutics. Mice that generate fully human antibodies with cognate heavy and light chains are known in the art. For the creation of these mice, it was necessary to disable the endogenous mouse immunoglobulin

genes so that the randomly integrated fully human transgenes would function as the expressed repertoire of immunoglobulins in the mouse. Such mice can make human antibodies suitable for use as human therapeutics, but these mice display substantial problems with their immune systems. These problems lead to several experimental hurdles, for example, the mice are impractical for generating sufficiently diverse antibody repertoires, require the use of extensive re-engineering fixes, provide a suboptimal clonal selection process likely due to incompatibility between human and mouse elements, and an unreliable source of large and diverse populations of human variable sequences needed to be truly useful for making human therapeutics.

[0004] Human antibody therapeutics are engineered based on desired characteristics with respect to selected antigens. Humanized mice are immunized with the selected antigens, and the immunized mice are used to generate antibody populations from which to identify high-affinity cognate heavy and light variable domains with desired binding characteristics. Some humanized mice, such as those having a humanization of just variable regions at endogenous mouse loci, generate populations of B cells that are similar in character and number to wild-type mouse B cell populations. As a result, an extremely large and diverse population of B cells is available in these mice from which to screen antibodies, reflecting a large number of different immunoglobulin rearrangements, to identify heavy and light variable domains with the most desirable characteristics.

[0005] However, not all antigens provoke an immune response that exhibits a very large number of rearrangements from a wide selection of variable (V) segments. That is, the human humoral immune response to certain antigens is apparently restricted. The restriction is reflected in clonal selection of B cells that express only certain V segments that bind that particular antigen with sufficiently high affinity and specificity. Some such antigens are clinically significant, *i.e.*, a number are well-known human pathogens. A presumption arises that the V segment expressed in the human immune response is a V segment that, in combination with a human D and a human J segment, is more likely to generate a useful high affinity antibody than a randomly selected V segment that has not been observed in a human antibody response to that antigen.

[0006] It is hypothesized that natural selection, over millennia of experience between humans and the pathogen, has selected the most efficient foundation or base from which to design its most effective weapon for neutralizing the pathogen--the selected V gene segment. There is a need in the art for superior antibodies that bind and/or neutralize antigens like the pathogens discussed above. There is a need to more rapidly generate useful sequences from selected V gene segments,

including polymorphic and/or somatically mutated selected V gene segments and to more rapidly generate useful populations of B cells having rearrangements of the V gene segments with various D and J gene segments, including somatically mutated versions thereof, and in particular rearrangements with unique and useful CDR3 regions. There is a need for improved biological systems, *e.g.*, non-human animals (such as, *e.g.*, mice, rats, rabbits, *etc.*) that can generate therapeutically useful antibody variable region sequences from selected V gene segments in increased number and diversity that, *e.g.*, can be achieved in existing modified animals, while at the same time reducing or eliminating deleterious changes that might result from the genetic modifications. There is a need for improved biological systems engineered to have a committed humoral immune system for clonally selecting antibody variable sequences derived from restricted, selected V gene segments, including but not limited to cognate human heavy and light chain variable domains, useful in the manufacture of human antibody-based therapeutics against selected antigens, including certain human pathogens. There remains a need in the art for making improved genetically modified mice that are useful in generating immunoglobulin sequences, including human antibody sequences, directed to the elimination of pathogens that burden the human population.

[0007] There is a need in the art for therapeutic antibodies that are capable of neutralizing viral antigens, *e.g.*, HIV and HCV, including antigen-specific antibodies containing heavy chains derived from a single human variable gene segment. There is also a need for further methods and non-human animals for making useful antibodies, including antibodies that comprise a repertoire of heavy chains derived from a single human V_H segment and having a diverse set of CDR sequences including heavy chains that express with cognate human light chains, and including restoration of unfavorable effects resulting from insertion of human genomic sequences into the genome of the non-human animals. Methods are needed for selecting CDRs for immunoglobulin-based binding proteins that provide an enhanced diversity of binding proteins from which to choose, and enhanced diversity of immunoglobulin variable domains, including compositions and methods for generating somatically mutated and clonally selected immunoglobulin variable domains for use, *e.g.*, in making human therapeutics.

SUMMARY

[0008] Genetically modified immunoglobulin loci are provided that comprise a restricted number of different heavy chain variable region gene segments (*i.e.*, V genes, V_H genes, V_H gene segments, or V gene segments), *e.g.*, no more than one,

two, or three different V genes; or no more than one V gene segment family member present, *e.g.*, in a single copy or in multiple copies and/or comprising one or more polymorphisms, and in various embodiments the loci lack a sequence that encodes an endogenous functional ADAM6 protein.

[0009] Loci are provided that are capable of rearranging and forming a gene encoding a heavy chain variable domain that is derived from a heavy chain V gene repertoire that is restricted, *e.g.*, that is a single V_H gene segment or selected from a plurality of polymorphic variants of the single V_H gene segment, wherein in various embodiments the loci lack an endogenous functional ADAM6 gene or functional fragment thereof.

[0010] Modified immunoglobulin loci include loci that lack an endogenous functional ADAM6 gene and comprise human immunoglobulin sequences are provided, *e.g.*, a human V segment operably linked to a human or (or human/non-human chimeric) non-human immunoglobulin constant sequence (and in operable linkage with, *e.g.*, a D and/or a J segment). Modified loci that comprise multiple copies of a single V_H gene segment, including wherein one or more of the copies comprises a polymorphic variant, and an ectopic nucleotide sequence that encodes an ADAM6 protein or fragment thereof that is functional in the non-human animal, are provided. Modified loci that comprise multiple copies of a single V_H segment, operably linked with one or more D segments and one or more J segments, operably linked to a non-human immunoglobulin constant sequence, *e.g.*, a mouse or rat or human sequence, are provided. Non-human animals comprising such humanized loci are also provided, wherein the non-human animals have wild-type fertility.

[0011] Non-human animals are provided that comprise an immunoglobulin heavy chain variable locus (*e.g.*, one a transgene or as an insertion or replacement at an endogenous non-human animal heavy chain variable locus) that comprises a single V_H segment operably linked to a D and/or J gene segment. In various embodiments, the single V_H gene segment is operably linked to one or more D and/or one or more J gene segments at the endogenous immunoglobulin heavy chain variable gene locus of the non-human animal. In various embodiments, the non-human animals further comprise an ectopic nucleotide sequence that encodes an ADAM6 protein or homolog or ortholog thereof that is functional in the male non-human animal that comprises the modified heavy chain locus. In various embodiments, the ectopic nucleotide sequence is contiguous with the single V_H segment, a D gene segment, or a J gene segment. In various embodiments, the ectopic nucleotide sequence is contiguous with a non-immunoglobulin sequence in the genome of the non-human animal. In one embodiment, the ectopic nucleotide

sequence is on the same chromosome as the modified heavy chain locus. In one embodiment, the ectopic nucleotide sequence is on a different chromosome as the modified heavy chain locus.

[0012] Non-human animals are provided that are modified at their immunoglobulin heavy chain variable region loci to delete all or substantially all (*e.g.*, all functional segments, or nearly all functional segments) endogenous immunoglobulin V_H segments and that comprise a human V_H1-69 segment (or a human V_H1-2 segment) operably linked to a D and J segment or a J segment at the endogenous immunoglobulin heavy chain variable region locus of the non-human animal. Non-human animals comprising such loci and that lack an endogenous ADAM6 gene(s) are also provided.

[0013] Methods are provided for making human immunoglobulin sequences in non-human animals. In various embodiments the human immunoglobulin sequences are derived from a repertoire of immunoglobulin V sequences that consist essentially of a single human V segment, *e.g.*, V_H1-69 or V_H1-2, and one or more D and J segments or one or more J segments. Methods for making human immunoglobulin sequences in non-human animals, tissues, and cells are provided, wherein the human immunoglobulin sequences bind a pathogen.

[0014] In one aspect, nucleic acid constructs, cells, embryos, mice, and methods are provided for making mice that comprise a modification that results in a nonfunctional endogenous mouse ADAM6 protein or ADAM6 gene (*e.g.*, a knockout of or a deletion in an endogenous ADAM6 gene), wherein the mice comprise a nucleic acid sequence that encodes an ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a male mouse. In one embodiment, the mice comprise an ectopic nucleotide sequence encoding a rodent ADAM6 protein or ortholog or homolog or functional fragment thereof; in a specific embodiment, the rodent ADAM6 protein is a mouse ADAM6 protein.

[0015] In one aspect, nucleic acid constructs, cells, embryos, mice, and methods are provided for making mice that comprise a modification of an endogenous mouse immunoglobulin locus, wherein the mice comprise an ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a male mouse. In one embodiment, the endogenous mouse immunoglobulin locus is an immunoglobulin heavy chain locus, and the modification reduces or eliminates ADAM6 activity of a cell or tissue of a male mouse. In one embodiment, the endogenous mouse immunoglobulin locus is an immunoglobulin heavy chain locus, and the modification maintains or sustains ADAM6 activity of a cell or tissue of a male mouse.

[0016] In one aspect, a modified immunoglobulin heavy chain locus is provided that comprises a heavy chain V segment repertoire that is restricted with respect to the identity of the V segment, and that comprises one or more D segments and one or more J segments, or one or more J segments. In one embodiment, the heavy chain V segment is a human segment. In one embodiment, the modified immunoglobulin heavy chain locus lacks an endogenous ADAM6 gene. In one embodiment, the modified heavy chain locus further comprises a nucleotide sequence that encodes an ADAM6 protein. In a specific embodiment, the nucleotide sequence is contiguous with the V, D and/or J gene segment at the modified immunoglobulin heavy chain locus.

[0017] In one embodiment, the modified locus is a non-human locus. In one embodiment, the non-human locus is modified with at least one human immunoglobulin sequence. In one embodiment, the non-human locus is modified with at least one human immunoglobulin sequence and a sequence that encodes an ADAM6 protein.

[0018] In one embodiment, the restriction is to one V segment family member. In one embodiment, the one V segment family member is present in two or more copies. In one embodiment, the one V segment family member is present as two or more variants (*e.g.,* two or more polymorphic forms of the V segment family member). In one embodiment, the one V segment is a human V segment family member. In one embodiment, the one V segment family member is present in a number of variants as is observed in the human population with respect to that variant. In one embodiment, the V segment family member is selected from Table 1. In one embodiment, the V segment family member is present in a number of variants as shown, for each V segment, in a number of alleles from 1 allele to the number of alleles shown in the right column of Table 1.

[0019] In one aspect, mice are provided that comprise an ectopic nucleotide sequence encoding a mouse ADAM6 or ortholog or homolog or functional fragment thereof; mice are also provided that comprise an endogenous nucleotide sequence encoding a mouse ADAM6 or ortholog or homolog or fragment thereof, and at least one genetic modification of a heavy chain immunoglobulin locus. In one embodiment, the endogenous nucleotide sequence encoding a mouse ADAM6 or ortholog or homolog or fragment thereof is located at an ectopic position as compared to an endogenous ADAM6 gene of a wild type mouse.

[0020] In one aspect, methods are provided for making mice that comprise a modification of an endogenous mouse immunoglobulin locus, wherein the mice comprise an ADAM6 protein or ortholog or homolog or fragment thereof that is

functional in a male mouse.

[0021] In one aspect, methods are provided for making mice that comprise a genetic modification of an immunoglobulin heavy chain locus, wherein application of the methods result in male mice that comprise a modified immunoglobulin heavy chain locus (or a deletion thereof), and the male mice are capable of generating offspring by mating. In one embodiment, the male mice are capable of producing sperm that can transit from a mouse uterus through a mouse oviduct to fertilize a mouse egg.

[0022] In one aspect, methods are provided for making mice that comprise a genetic modification of an immunoglobulin heavy chain locus, wherein application of the methods result in male mice that comprise a modified immunoglobulin heavy chain locus (or a deletion thereof), and the male mice exhibit a reduction in fertility, and the mice comprise a genetic modification that restores in whole or in part the reduction in fertility. In various embodiments, the reduction in fertility is characterized by an inability of the sperm of the male mice to migrate from a mouse uterus through a mouse oviduct to fertilize a mouse egg. In various embodiments, the reduction in fertility is characterized by sperm that exhibit an *in vivo* migration defect. In various embodiments, the genetic modification that restores in whole or in part the reduction in fertility is a nucleic acid sequence encoding a mouse ADAM6 gene or ortholog or homolog or fragment thereof that is functional in a male mouse.

[0023] In one embodiment, the genetic modification comprises replacing endogenous immunoglobulin heavy chain variable loci with a restricted number, *e.g.*, no more than one, two or three different heavy chain variable (V_H) gene segments, one or more heavy chain diversity (D_H) gene segments and one or more heavy chain joining (J_H) gene segments of another species (*e.g.*, a non-mouse species). In one embodiment, the genetic modification comprises insertion of a single orthologous immunoglobulin V_H gene segment, at least one D_H gene segment, and at least one J_H gene segment into endogenous immunoglobulin heavy chain variable loci. In a specific embodiment, the species is human. In one embodiment, the genetic modification comprises deletion of an endogenous immunoglobulin heavy chain variable locus in whole or in part, wherein the deletion results in a loss of endogenous ADAM6 function. In a specific embodiment, the loss of endogenous ADAM6 function is associated with a reduction in fertility in male mice. In one embodiment, the genetic modification comprises inactivation of an endogenous immunoglobulin heavy chain variable locus in whole or in part, wherein the deletion does not result in a loss of endogenous ADAM6 function. Inactivation may include replacement or deletion of one or more endogenous gene segments resulting in an

endogenous immunoglobulin heavy chain locus that is substantially incapable of rearrangement to encode a heavy chain of an antibody that comprises endogenous gene segments. Inactivation may include other modifications that render the endogenous immunoglobulin heavy chain locus incapable of rearranging to encode the heavy chain of an antibody, wherein the modification does not include replacement or deletion of endogenous gene segments. Exemplary modifications include chromosomal inversions and/or translocations mediated by molecular techniques, *e.g.*, using precise placement of site-specific recombination sites (*e.g.*, Cre-lox technology).

[0024] In one embodiment, the genetic modification comprises inserting into the genome of the mouse a DNA fragment containing a restricted number, *e.g.*, no more than one, two or three different heavy chain variable (V_H) gene segments, one or more heavy chain diversity (D_H) gene segments and one or more heavy chain joining (J_H) gene segments of another species (*e.g.*, a non-mouse species) operably linked to one or more constant region sequences (*e.g.*, an IgM and/or an IgG gene). In one embodiment, the DNA fragment is capable of undergoing rearrangement to form a sequence that encodes a heavy chain of an antibody. In one embodiment, the genetic modification comprises insertion of a single orthologous immunoglobulin V_H gene segment, at least one D_H gene segment, and at least one J_H gene segment into the genome of the mouse. In a specific embodiment, the species is human. In one embodiment, the genetic modification comprises deletion of an endogenous immunoglobulin heavy chain variable locus in whole or in part to render the endogenous immunoglobulin heavy chain locus nonfunctional, wherein the deletion further results in a loss of endogenous ADAM6 function. In a specific embodiment, the loss of endogenous ADAM6 function is associated with a reduction in fertility in male mice.

[0025] In one aspect, mice are provided that comprise a modification that reduces or eliminates mouse ADAM6 expression from an endogenous ADAM6 allele such that a male mouse having the modification exhibits a reduced fertility (*e.g.*, a highly reduced ability to generate offspring by mating), or is essentially infertile, due to the reduction or elimination of endogenous ADAM6 function, wherein the mice further comprise an ectopic ADAM6 sequence or homolog or ortholog or functional fragment thereof. In one aspect, the modification that reduces or eliminates mouse ADAM6 expression is a modification (*e.g.*, an insertion, a deletion, a replacement, *etc.*) in a mouse immunoglobulin locus. In one embodiment, the immunoglobulin locus is an immunoglobulin heavy chain locus.

[0026] In one embodiment, the reduction or loss of ADAM6 function comprises

an inability or substantial inability of the mouse to produce sperm that can travel from a mouse uterus through a mouse oviduct to fertilize a mouse egg. In a specific embodiment, at least about 95%, 96%, 97%, 98%, or 99% of the sperm cells produced in an ejaculate volume of the mouse are incapable of traversing through an oviduct in vivo following copulation and fertilizing a mouse ovum.

[0027] In one embodiment, the reduction or loss of ADAM6 function comprises an inability to form or substantial inability to form a complex of ADAM2 and/or ADAM3 and/or ADAM6 on a surface of a sperm cell of the mouse. In one embodiment, the loss of ADAM6 function comprises a substantial inability to fertilize a mouse egg by copulation with a female mouse.

[0028] In one aspect, a mouse is provided that lacks a functional endogenous ADAM6 gene, and comprises a protein (or an ectopic nucleotide sequence that encodes a protein) that confers ADAM6 functionality on the mouse. In one embodiment, the mouse is a male mouse and the functionality comprises enhanced fertility as compared with a mouse that lacks a functional endogenous ADAM6 gene.

[0029] In one embodiment, the protein is encoded by a genomic sequence located within an immunoglobulin locus in the germline of the mouse. In a specific embodiment, the immunoglobulin locus is a heavy chain locus. In another specific embodiment, the heavy chain locus comprises a single human V_H, at least one human D_H and at least one human J_H gene segment. In another specific embodiment, the heavy chain locus comprises one human V_H gene segment, 27 human D_H gene segments, and six human J_H gene segments. In one embodiment, the ectopic protein is encoded by a genomic sequence located within a non-immunoglobulin locus in the germline of the mouse. In one embodiment, the non-immunoglobulin locus is a transcriptionally active locus. In a specific embodiment, the transcriptionally active locus is the ROSA26 locus. In a specific embodiment, the transcriptionally active locus is associated with tissue-specific expression. In one embodiment, the tissue-specific expression is present in reproductive tissues. In one embodiment, the protein is encoded by a genomic sequence randomly inserted into the germline of the mouse.

[0030] In one embodiment, the mouse comprises a human or chimeric human/mouse or chimeric human/rat light chain (*e.g.*, human variable, mouse or rat constant) and a chimeric human variable/mouse or rat constant heavy chain. In a specific embodiment, the mouse comprises a transgene that comprises a chimeric human variable/rat or mouse constant light chain gene operably linked to a transcriptionally active promoter, *e.g.*, a ROSA26 promoter. In a further specific embodiment, the chimeric human/mouse or rat light chain transgene comprises a

rearranged human light chain variable region sequence in the germline of the mouse.

[0031] In one embodiment, the ectopic nucleotide sequence is located within an immunoglobulin locus in the germline of the mouse. In a specific embodiment, the immunoglobulin locus is a heavy chain locus. In one embodiment, the heavy chain locus comprises a single human V_H, at least one human D_H and at least one human J_H gene segment. In a specific embodiment, the heavy chain locus comprises a single human V_H, 27 human D_H gene segments and six human J_H gene segments. In one embodiment, the ectopic nucleotide sequence is located within a non-immunoglobulin locus in the germline of the mouse. In one embodiment, the non-immunoglobulin locus is a transcriptionally active locus. In a specific embodiment, the transcriptionally active locus is the ROSA26 locus. In one embodiment, the ectopic nucleotide sequence is positioned randomly inserted into the germline of the mouse.

[0032] In one aspect, a mouse is provided that lacks a functional endogenous ADAM6 gene, wherein the mouse comprises an ectopic nucleotide sequence that complements the loss of mouse ADAM6 function. In one embodiment, the ectopic nucleotide sequence confers upon the mouse an ability to produce offspring that is comparable to a corresponding wild-type mouse that contains a functional endogenous ADAM6 gene. In one embodiment, the sequence confers upon the mouse an ability to form a complex of ADAM2 and/or ADAM3 and/or ADAM6 on the surface of sperm cell of the mouse. In one embodiment, the sequence confers upon the mouse an ability to travel from a mouse uterus through a mouse oviduct to a mouse ovum to fertilize the ovum.

[0033] In one embodiment, the mouse lacking the functional endogenous ADAM6 gene and comprising the ectopic nucleotide sequence produces at least about 50%, 60%, 70%, 80%, or 90% of the number of litters a wild-type mouse of the same age and strain produces in a six-month time period.

[0034] In one embodiment, the mouse lacking the functional endogenous ADAM6 gene and comprising the ectopic nucleotide sequence produces at least about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 4-fold, about 6-fold, about 7-fold, about 8-fold, or about 10-fold or more progeny when bred over a six-month time period than a mouse of the same age and the same or similar strain that lacks the functional endogenous ADAM6 gene and that lacks the ectopic nucleotide sequence that is bred over substantially the same time period and under substantially the same conditions.

[0035] In one embodiment, the mouse lacking the functional endogenous ADAM6 gene and comprising the ectopic nucleotide sequence produces an average

of at least about 2-fold, 3-fold, or 4-fold higher number of pups per litter in a 4- or 6-month breeding period than a mouse that lacks the functional endogenous ADAM6 gene and that lacks the ectopic nucleotide sequence, and that is bred for the same period of time.

[0036] In one embodiment, the mouse lacking the functional endogenous ADAM6 gene and comprising the ectopic nucleotide sequence is a male mouse, and the male mouse produces sperm that when recovered from oviducts at about 5-6 hours post-copulation reflects an oviduct migration that is at least 10-fold, at least 20-fold, at least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, 100-fold, 110-fold, or 120-fold or higher than a mouse that lacks the functional endogenous ADAM6 gene and that lacks the ectopic nucleotide sequence.

[0037] In one embodiment, the mouse lacking the functional endogenous ADAM6 gene and comprising the ectopic nucleotide sequence when copulated with a female mouse generates sperm that is capable of traversing the uterus and entering and traversing the oviduct within about 6 hours at an efficiency that is about equal to sperm from a wild-type mouse.

[0038] In one embodiment, the mouse lacking the functional endogenous ADAM6 gene and comprising the ectopic nucleotide sequence produces about 1.5-fold, about 2-fold, about 3-fold, or about 4-fold or more litters in a comparable period of time than a mouse that lacks the functional ADAM6 gene and that lacks the ectopic nucleotide sequence.

[0039] In one aspect, a mouse comprising in its germline a non-mouse nucleic acid sequence that encodes an immunoglobulin protein is provided, wherein the non-mouse immunoglobulin sequence comprises an insertion of a mouse ADAM6 gene or homolog or ortholog or functional fragment thereof. In one embodiment, the non-mouse immunoglobulin sequence comprises a human immunoglobulin sequence. In one embodiment, the sequence comprises a human immunoglobulin heavy chain sequence. In one embodiment, the sequence comprises a human immunoglobulin light chain sequence. In one embodiment, the sequence comprises a single V_H gene segment, one or more D_H gene segments, and one or more J_H gene segments; in one embodiment, the sequence comprises one or more V_L gene segments and one or more J_L gene segments. In one embodiment, the single V_H, one or more D_H, and one or more J_H gene segments, or one or more V_L and J_L gene segments, are not rearranged. In one embodiment, the single V_H, one or more D_H, and one or more J_H gene segments, or one or more V_L and J_L gene segments, are rearranged. In one embodiment, following rearrangement of the single V_H, one or more D_H, and one or

more J_H gene segments, or one or more V_L and J_L gene segments, the mouse comprises in its genome at least one nucleic acid sequence encoding a mouse ADAM6 gene or homolog or ortholog or functional fragment thereof. In one embodiment, following rearrangement the mouse comprises in its genome at least two nucleic acid sequences encoding a mouse ADAM6 gene or homolog or ortholog or functional fragment thereof. In one embodiment, following rearrangement the mouse comprises in its genome at least one nucleic acid sequence encoding a mouse ADAM6 gene or homolog or ortholog or functional fragment thereof. In one embodiment, the mouse comprises the ADAM6 gene or homolog or ortholog or functional fragment thereof in a B cell. In one embodiment, the mouse comprises the ADAM6 gene or homolog or ortholog or functional fragment thereof in a non-B cell.

[0040] In one aspect, mice are provided that express a human immunoglobulin heavy chain variable region or functional fragment thereof from an endogenous mouse immunoglobulin heavy chain locus, wherein the mice comprise an ADAM6 activity that is functional in a male mouse. In one embodiment, the human immunoglobulin heavy chain variable region comprises a polymorphic human V_H gene segment. In one embodiment, the human immunoglobulin heavy chain variable region comprises a human V_H1-69 gene segment. In one embodiment, the human immunoglobulin heavy chain variable region comprises a human V_H1-2 gene segment.

[0041] In one embodiment, the male mice comprise a single unmodified endogenous ADAM6 allele or ortholog of homolog or functional fragment thereof at an endogenous ADAM6 locus.

[0042] In one embodiment, the male mice comprise an ectopic mouse ADAM6 sequence or homolog or ortholog or functional fragment thereof that encodes a protein that confers ADAM6 function.

[0043] In one embodiment, the male mice comprise an ADAM6 sequence or homolog or ortholog or functional fragment thereof at a location in the mouse genome that approximates the location of the endogenous mouse ADAM6 allele, *e.g.*, 3' of a V gene segment sequence and 5' of an initial D gene segment. In a specific embodiment, the male mice comprise an ADAM6 sequence or homolog or ortholog or functional fragment thereof 3' of a human V_H gene segment and 5' of a human D_H gene segment. In another specific embodiment, the male mice comprise an ADAM6 sequence or homolog or ortholog or functional fragment thereof 5' of a human V_H gene segment. In another specific embodiment, the male mice comprise an ADAM6 sequence or homolog or ortholog or functional fragment thereof 5' of a chimeric heavy chain locus comprising a single human V_H gene segment, one or

more D_H gene segments, and one or more J_H gene segments. In one embodiment, the chimeric heavy chain locus comprises a human V_H1-69 gene segment, 27 human D_H gene segments, and six human J_H gene segments. In one embodiment, the chimeric heavy chain locus comprises a human V_H1-2 gene segment, 27 human D_H gene segments, and six human J_H gene segments.

[0044] In one embodiment, the male mice comprise an ADAM6 sequence or homolog or ortholog or functional fragment thereof flanked upstream, downstream, or upstream and downstream (with respect to the direction of transcription of the ADAM6 sequence) of a nucleic acid sequence encoding an immunoglobulin variable gene segment or an immunoglobulin diversity gene segment. In a specific embodiment, the immunoglobulin variable gene segment is a human gene segment. In one embodiment, the immunoglobulin variable gene segment is a human gene segment, and the sequence encoding the mouse ADAM6 or ortholog or homolog or fragment thereof functional in a mouse is between human V_H gene segments; in one embodiment, the mouse comprises one human V_H gene segment, and the sequence is at a position 5' of the V_H gene segment; in one embodiment, the sequence is at a position 3' of the V_H gene segment; in one embodiment, the sequence is at a position between the V_H gene segment and the first D_H gene segment. In a specific embodiment, the D_H gene segment is the first D_H gene segment. In one embodiment, the mouse comprises two V_H gene segments, and the sequence is at a position between the two V_H gene segments; in one embodiment, the sequence is at a position between a V_H gene segment and a D_H gene segment. In a specific embodiment, the D_H gene segment is the first D_H gene segment.

[0045] In one embodiment, the male mice comprise an ADAM6 sequence or homolog or ortholog or functional fragment thereof that is located at a position in an endogenous immunoglobulin locus that is the same or substantially the same as in a wild type male mouse. In a specific embodiment, the endogenous locus is incapable of encoding the heavy chain of an antibody. In a specific embodiment, the endogenous locus is positioned at a location in the genome of the male mouse that renders it incapable of encoding the heavy chain of an antibody. In various embodiments, the male mice comprise an ADAM6 sequence located on the same chromosome as human immunoglobulin gene segments and the ADAM6 sequence encodes a functional ADAM6 protein.

[0046] In one aspect, a male mouse is provided that comprises a nonfunctional endogenous ADAM6 gene, or a deletion of an endogenous ADAM6 gene, in its germline; wherein sperm cells of the mouse are capable of transiting an oviduct of a female mouse and fertilizing an egg. In one embodiment, the mice comprise an

extrachromosomal copy of a mouse ADAM6 gene or ortholog or homolog or functional fragment thereof that is functional in a male mouse. In one embodiment, the mice comprise an ectopic mouse ADAM6 gene or ortholog or homolog or functional fragment thereof that is functional in a male mouse.

[0047] In one aspect, mice are provided that comprise a genetic modification that reduces endogenous mouse ADAM6 function, wherein the mouse comprises at least some ADAM6 functionality provided either by an endogenous unmodified allele that is functional in whole or in part (*e.g.*, a heterozygote), or by expression from an ectopic sequence that encodes an ADAM6 or an ortholog or homolog or functional fragment thereof that is functional in a male mouse.

[0048] In one embodiment, the mice comprise ADAM6 function sufficient to confer upon male mice the ability to generate offspring by mating, as compared with male mice that lack a functional ADAM6. In one embodiment, the ADAM6 function is conferred by the presence of an ectopic nucleotide sequence that encodes a mouse ADAM6 or a homolog or ortholog or functional fragment thereof. In one embodiment, the ADAM6 function is conferred by an endogenous ADAM6 gene present in an endogenous immunoglobulin locus, wherein the endogenous immunoglobulin locus is incapable of encoding the heavy chain of an antibody. ADAM6 homologs or orthologs or fragments thereof that are functional in a male mouse include those that restore, in whole or in part, the loss of ability to generate offspring observed in a male mouse that lacks sufficient endogenous mouse ADAM6 activity, *e.g.*, the loss in ability observed in an ADAM6 knockout mouse. In this sense ADAM6 knockout mice include mice that comprise an endogenous locus or fragment thereof, but that is not functional, *i.e.*, that does not express ADAM6 (ADAM6a and/or ADAM6b) at all, or that expresses ADAM6 (ADAM6a and/or ADAM6b) at a level that is insufficient to support an essentially normal ability to generate offspring of a wild-type male mouse. The loss of function can be due, *e.g.*, to a modification in a structural gene of the locus (*i.e.*, in an ADAM6a or ADAM6b coding region) or in a regulatory region of the locus (*e.g.*, in a sequence 5' to the ADAM6a gene, or 3' of the ADAM6a or ADAM6b coding region, wherein the sequence controls, in whole or in part, transcription of an ADAM6 gene, expression of an ADAM6 RNA, or expression of an ADAM6 protein). In various embodiments, orthologs or homologs or fragments thereof that are functional in a male mouse are those that enable a sperm of a male mouse (or a majority of sperm cells in the ejaculate of a male mouse) to transit a mouse oviduct and fertilize a mouse ovum.

[0049] In one embodiment, male mice that express the human immunoglobulin variable region or functional fragment thereof comprise sufficient ADAM6 activity to

confer upon the male mice the ability to generate offspring by mating with female mice and, in one embodiment, the male mice exhibit an ability to generate offspring when mating with female mice that is in one embodiment at least 25%, in one embodiment, at least 30%, in one embodiment at least 40%, in one embodiment at least 50%, in one embodiment at least 60%, in one embodiment at least 70%, in one embodiment at least 80%, in one embodiment at least 90%, and in one embodiment about the same as, that of mice with one or two endogenous unmodified ADAM6 alleles.

[0050] In one embodiment male mice express sufficient ADAM6 (or an ortholog or homolog or functional fragment thereof) to enable a sperm cell from the male mice to traverse a female mouse oviduct and fertilize a mouse egg.

[0051] In one embodiment, the ADAM6 functionality is conferred by a nucleic acid sequence that is contiguous with a mouse chromosomal sequence (*e.g.*, the nucleic acid is randomly integrated into a mouse chromosome; or placed at a specific location, *e.g.*, by targeting the nucleic acid to a specific location, *e.g.*, by site-specific recombinase-mediated (*e.g.*, Cre-mediated) insertion or homologous recombination). In one embodiment, the ADAM6 sequence is present on a nucleic acid that is distinct from a chromosome of the mouse (*e.g.*, the ADAM6 sequence is present on an episome, *i.e.*, extrachromosomally, *e.g.*, in an expression construct, a vector, a YAC, a transchromosome, *etc.*).

[0052] In one aspect, genetically modified mice and cells are provided that comprise a modification of an endogenous immunoglobulin heavy chain locus, wherein the mice express at least a portion of an immunoglobulin heavy chain sequence, *e.g.*, at least a portion of a human sequence, wherein the mice comprise an ADAM6 activity that is functional in a male mouse. In one embodiment, the modification reduces or eradicates ADAM6 activity of the mouse. In one embodiment, the mouse is modified such that both alleles that encode ADAM6 activity are either absent or express an ADAM6 that does not substantially function to support normal mating in a male mouse. In one embodiment, the mouse further comprises an ectopic nucleic acid sequence encoding a mouse ADAM6 or ortholog or homolog or functional fragment thereof. In one embodiment, the modification maintains ADAM6 activity of the mouse and renders an endogenous immunoglobulin heavy chain locus incapable of encoding a heavy chain of an antibody. In a specific embodiment, the modification includes chromosomal inversions and or translocations that render the endogenous immunoglobulin heavy chain locus incapable of encoding a heavy chain of an antibody.

[0053] In one aspect, genetically modified mice and cells are provided that

comprise a modification of an endogenous immunoglobulin heavy chain locus, wherein the modification reduces or eliminates ADAM6 activity expressed from an ADAM6 sequence of the locus, and wherein the mice comprise an ADAM6 protein or ortholog or homolog or functional fragment thereof. In various embodiments, the ADAM6 protein or fragment thereof is encoded by an ectopic ADAM6 sequence. In various embodiments, the ADAM6 protein or fragment thereof is expressed from an endogenous ADAM6 allele. In various embodiments, the mouse comprises a first immunoglobulin heavy chain allele comprises a first modification that reduces or eliminates expression of a functional ADAM6 from the first immunoglobulin heavy chain allele, and the mouse comprises a second immunoglobulin heavy chain allele that comprises a second modification that does not substantially reduce or does not eliminate expression of a functional ADAM6 from the second immunoglobulin heavy chain allele.

[0054] In one embodiment, the second modification is located 3' (with respect to the transcriptional directionality of the mouse V gene segment) of a final mouse V gene segment and located 5' (with respect to the transcriptional directionality of the constant sequence) of a mouse (or chimeric human/mouse) immunoglobulin heavy chain constant gene or fragment thereof (*e.g.*, a nucleic acid sequence encoding a human and/or mouse: C_H1 and/or hinge and/or C_H2 and/or C_H3).

[0055] In one embodiment, the modification is at a first immunoglobulin heavy chain allele at a first locus that encodes a first ADAM6 allele, and the ADAM6 function results from expression of an endogenous ADAM6 at a second immunoglobulin heavy chain allele at a second locus that encodes a functional ADAM6, wherein the second immunoglobulin heavy chain allele comprises at least one modification of a V, D, and/or J gene segment. In a specific embodiment, the at least one modification of the V, D, and or J gene segment is a deletion, a replacement with a single human V_H, one or more D_H, and/or one or more J_H gene segments, a replacement with a camelid V_H (or V_{HH}), D_H, and/or J_H gene segment, a replacement with a humanized or camelized V_H (or V_{HH}), D_H, and/or J_H gene segment, a replacement of a heavy chain sequence with a light chain sequence, and a combination thereof. In one embodiment, the at least one modification is the deletion of one or more V_H, D_H, and/or J_H gene segments and a replacement with one or more V_L and/or J_L gene segments (*e.g.*, a human V_L and/or J_L gene segment) at the heavy chain locus.

[0056] In one embodiment, the modification is at a first immunoglobulin heavy chain allele at a first locus and a second immunoglobulin heavy chain allele at a second locus, and the ADAM6 function results from expression of an ectopic ADAM6

at a non-immunoglobulin locus in the germline of the mouse. In a specific embodiment, the non-immunoglobulin locus is the ROSA26 locus. In a specific embodiment, the non-immunoglobulin locus is transcriptionally active in reproductive tissue.

[0057] In one embodiment, the modification is at a first immunoglobulin heavy chain allele at a first locus and a second immunoglobulin heavy chain allele at a second locus, and the ADAM6 function results from expression of an ectopic ADAM6 at the first immunoglobulin heavy chain allele. In one embodiment, the modification is at a first immunoglobulin heavy chain allele at a first locus and a second immunoglobulin heavy chain allele at a second locus, and the ADAM6 function results from expression of an ectopic ADAM6 at the second immunoglobulin heavy chain allele.

[0058] In one aspect, a mouse comprising a heterozygous or a homozygous knockout of ADAM6 is provided. In one embodiment, the mouse further comprises a modified immunoglobulin sequence that is a human or a humanized immunoglobulin sequence, or a camelid or camelized human or mouse immunoglobulin sequence. In one embodiment, the modified immunoglobulin sequence is present at the endogenous mouse heavy chain immunoglobulin locus. In one embodiment, the modified immunoglobulin sequence comprises a human heavy chain variable gene sequence at an endogenous mouse immunoglobulin heavy chain locus. In one embodiment, the human heavy chain variable gene sequence replaces an endogenous mouse heavy chain variable gene sequence at the endogenous mouse immunoglobulin heavy chain locus.

[0059] In one aspect, a mouse incapable of expressing a functional endogenous mouse ADAM6 from an endogenous mouse ADAM6 locus is provided. In one embodiment, the mouse comprises an ectopic nucleic acid sequence that encodes an ADAM6, or functional fragment thereof, that is functional in the mouse. In a specific embodiment, the ectopic nucleic acid sequence encodes a protein that rescues a loss in the ability to generate offspring exhibited by a male mouse that is homozygous for an ADAM6 knockout. In a specific embodiment, the ectopic nucleic acid sequence encodes a mouse ADAM6 protein.

[0060] In one aspect, a mouse is provided that lacks a functional endogenous ADAM6 locus, and that comprises an ectopic nucleic acid sequence that confers upon the mouse ADAM6 function. In one embodiment, the nucleic acid sequence comprises an endogenous mouse ADAM6 sequence or functional fragment thereof. In one embodiment, the endogenous mouse ADAM6 sequence comprises ADAM6a- and ADAM6b-encoding sequence located in a wild-type mouse between the 3'-most

mouse immunoglobulin heavy chain V gene segment (V_H) and the 5'-most mouse immunoglobulin heavy chain D gene segment (D_H).

[0061] In one embodiment, the nucleic acid sequence comprises a sequence encoding mouse ADAM6a or functional fragment thereof and/or a sequence encoding mouse ADAM6b or functional fragment thereof, wherein the ADAM6a and/or ADAM6b or functional fragment(s) thereof is operably linked to a promoter. In one embodiment, the promoter is a human promoter. In one embodiment, the promoter is the mouse ADAM6 promoter. In a specific embodiment, the ADAM6 promoter comprises sequence located between the first codon of the first ADAM6 gene closest to the mouse 5'-most D_H gene segment and the recombination signal sequence of the 5'-most D_H gene segment, wherein 5' is indicated with respect to direction of transcription of the mouse immunoglobulin genes. In one embodiment, the promoter is a viral promoter. In a specific embodiment, the viral promoter is a cytomegalovirus (CMV) promoter. In one embodiment, the promoter is a ubiquitin promoter.

[0062] In one embodiment, the promoter is an inducible promoter. In one embodiment, the inducible promoter regulates expression in non-reproductive tissues. In one embodiment, the inducible promoter regulates expression in reproductive tissues. In a specific embodiment, the expression of the mouse ADAM6a and/or ADAM6b sequences or functional fragments(s) thereof is developmentally regulated by the inducible promoter in reproductive tissues.

[0063] In one embodiment, the mouse ADAM6a and/or ADAM6b are selected from the ADAM6a of SEQ ID NO: 1 and/or ADAM6b of sequence SEQ ID NO: 2.

[0064] In one embodiment, the mouse ADAM6 promoter is a promoter of SEQ ID NO: 3. In a specific embodiment, the mouse ADAM6 promoter comprises the nucleic acid sequence of SEQ ID NO: 3 directly upstream (with respect to the direction of transcription of ADAM6a) of the first codon of ADAM6a and extending to the end of SEQ ID NO: 3 upstream of the ADAM6 coding region. In another specific embodiment, the ADAM6 promoter is a fragment extending from within about 5 to about 20 nucleotides upstream of the start codon of ADAM6a to about 0.5kb, 1kb, 2kb, or 3kb or more upstream of the start codon of ADAM6a.

[0065] In one embodiment, the mouse ADAM6 promoter is a promoter of SEQ ID NO: 73. In a specific embodiment, the mouse ADAM6 promoter comprises the nucleic acid sequence of SEQ ID NO: 73 directly upstream (with respect to the direction of transcription of ADAM6a) of the first codon of ADAM6a and extending to the end of SEQ ID NO: 73 upstream of the ADAM6 coding region. In another specific embodiment, the ADAM6 promoter is a fragment extending from within about

5 to about 20 nucleotides upstream of the start codon of ADAM6a to about 0.5kb, 1kb, 2kb, or 3kb or more upstream of the start codon of ADAM6a.

[0066] In one embodiment, the mouse ADAM6 promoter is a promoter of SEQ ID NO: 77. In a specific embodiment, the mouse ADAM6 promoter comprises the nucleic acid sequence of SEQ ID NO: 77 directly upstream (with respect to the direction of transcription of ADAM6a) of the first codon of ADAM6a and extending to the end of SEQ ID NO: 77 upstream of the ADAM6 coding region. In another specific embodiment, the ADAM6 promoter is a fragment extending from within about 5 to about 20 nucleotides upstream of the start codon of ADAM6a to about 0.5kb, 1kb, 2kb, or 3kb or more upstream of the start codon of ADAM6a.

[0067] In one embodiment, the nucleic acid sequence comprises SEQ ID NO: 3 or a fragment thereof that when placed into a mouse that is infertile or that has low fertility due to a lack of ADAM6, improves fertility or restores fertility to about a wild-type fertility. In one embodiment, SEQ ID NO: 3 or a fragment thereof confers upon a male mouse the ability to produce a sperm cell that is capable of traversing a female mouse oviduct in order to fertilize a mouse egg.

[0068] In one embodiment, the nucleic acid sequence comprises SEQ ID NO: 73 or a fragment thereof that when placed into a mouse that is infertile or that has low fertility due to a lack of ADAM6, improves fertility or restores fertility to about a wild-type fertility. In one embodiment, SEQ ID NO: 73 or a fragment thereof confers upon a male mouse the ability to produce a sperm cell that is capable of traversing a female mouse oviduct in order to fertilize a mouse egg.

[0069] In one embodiment, the nucleic acid sequence comprises SEQ ID NO: 77 or a fragment thereof that when placed into a mouse that is infertile or that has low fertility due to a lack of ADAM6, improves fertility or restores fertility to about a wild-type fertility. In one embodiment, SEQ ID NO: 77 or a fragment thereof confers upon a male mouse the ability to produce a sperm cell that is capable of traversing a female mouse oviduct in order to fertilize a mouse egg.

[0070] In one aspect, a mouse is provided that comprises a deletion of an endogenous nucleotide sequence that encodes an ADAM6 protein, a replacement of an endogenous mouse V_H gene segment with a human V_H gene segment, and an ectopic nucleotide sequence that encodes a mouse ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a male mouse.

[0071] In one embodiment, the mouse comprises an immunoglobulin heavy chain locus that comprises a deletion of an endogenous immunoglobulin locus nucleotide sequence that comprises an endogenous ADAM6 gene, comprises a nucleotide sequence encoding one or more human immunoglobulin gene segments,

and wherein the ectopic nucleotide sequence encoding the mouse ADAM6 protein is within or directly adjacent to the nucleotide sequence encoding the one or more human immunoglobulin gene segments.

[0072] In one embodiment, the mouse comprises a replacement of all or substantially all endogenous V_H gene segments with a nucleotide sequence encoding a single human V_H gene segment, and the ectopic nucleotide sequence encoding the mouse ADAM6 protein is within, or directly adjacent to, the nucleotide sequence encoding the single human V_H gene segment. In one embodiment, the mouse further comprises a replacement of one or more endogenous D_H gene segments with one or more human D_H gene segments at the endogenous D_H gene locus. In one embodiment, the mouse further comprises a replacement of one or more endogenous J_H gene segments with one or more human J_H gene segments at the endogenous J_H gene locus. In one embodiment, the mouse comprises a replacement of all or substantially all endogenous V_H, D_H, and J_H gene segments and a replacement at the endogenous V_H, D_H, and J_H gene loci with a single human V_H, one or more human D_H, and one or more human J_H gene segments, wherein the mouse comprises an ectopic sequence encoding a mouse ADAM6 protein. In a specific embodiment, the ectopic sequence encoding the mouse ADAM6 protein is placed upstream or 5' of the single human V_H gene segment. In another specific embodiment, the ectopic sequence encoding the mouse ADAM6 protein is placed downstream or 3' of the single human V_H gene segment. In another specific embodiment, the ectopic sequence encoding the mouse ADAM6 protein is placed between the single human V_H gene segment and the first human D_H gene segment present. In another specific embodiment, the mouse comprises a deletion of all or substantially all mouse V_H gene segments, and a replacement with a single human V_H gene segment, and the ectopic nucleotide sequence encoding the mouse ADAM6 protein is placed downstream of human gene segment V_H1-69 and upstream of human gene segment D_H1-1. In another specific embodiment, the mouse comprises a deletion of all or substantially all mouse V_H gene segments, and a replacement with a single human V_H gene segment, and the ectopic nucleotide sequence encoding the mouse ADAM6 protein is placed downstream of human gene segment V_H1-2 and upstream of human gene segment D_H1-1.

[0073] In a specific embodiment, the mouse comprises a replacement of all or substantially all endogenous V_H gene segments with a nucleotide sequence encoding a single V_H gene segments, and the ectopic nucleotide sequence encoding the mouse ADAM6 protein is within, or directly adjacent to, the nucleotide sequence encoding the single human V_H gene segment.

[0074] In one embodiment, the ectopic nucleotide sequence that encodes the mouse ADAM6 protein is present on a transgene in the genome of the mouse. In one embodiment, the ectopic nucleotide sequence that encodes the mouse ADAM6 protein is present extrachromosomally in the mouse.

[0075] In one aspect, a mouse is provided that comprises a modification of an endogenous immunoglobulin heavy chain locus, wherein the mouse expresses a B cell that comprises a rearranged immunoglobulin sequence operably linked to a heavy chain constant region gene sequence, and the B cell comprises in its genome (*e.g.*, on a B cell chromosome) a gene encoding an ADAM6 or ortholog or homolog or fragment thereof that is functional in a male mouse. In one embodiment, the rearranged immunoglobulin sequence operably linked to the heavy chain constant region gene sequence comprises a human heavy chain V, D, and/or J sequence; a mouse heavy chain V, D, and/or J sequence; a human or mouse light chain V and/or J sequence. In one embodiment, the heavy chain constant region gene sequence comprises a human or a mouse heavy chain sequence selected from the group consisting of a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof.

[0076] In one aspect, a mouse is provided that comprises a functionally silenced endogenous immunoglobulin heavy chain locus, wherein ADAM6 function is maintained in the mouse, and further comprises an insertion of one or more human immunoglobulin gene segments, wherein the one or more human immunoglobulin gene segments include a single human V_H gene segment, one or more human D_H gene segments, and one or more human J_H gene segments. In one embodiment, the one or more human immunoglobulin gene segments includes a human V_H1-69 gene segment, 27 human D_H gene segments, and six human J_H gene segments. In one embodiment, the one or more human immunoglobulin gene segments include a human V_H1-2 gene segment, 27 human D_H gene segments, and six human J_H gene segments.

[0077] In one aspect, a genetically modified mouse is provided, wherein the mouse comprises a functionally silenced immunoglobulin light chain gene, and further comprises a replacement of one or more endogenous immunoglobulin heavy chain variable region gene segments with a single human immunoglobulin heavy chain variable region gene segment, wherein the mouse lacks a functional endogenous ADAM6 locus, and wherein the mouse comprises an ectopic nucleotide sequence that expresses a mouse ADAM6 protein or an ortholog or homolog or fragment thereof that is functional in a male mouse.

[0078] In one aspect, a mouse is provided that lacks a functional endogenous mouse ADAM6 locus or sequence and that comprises an ectopic nucleotide

sequence encoding a mouse ADAM6 locus or functional fragment of a mouse ADAM6 locus or sequence, wherein the mouse is capable of mating with a mouse of the opposite sex to produce a progeny that comprises the ectopic ADAM6 locus or sequence. In one embodiment, the mouse is male. In one embodiment, the mouse is female.

[0079] In one aspect, a genetically modified mouse is provided, wherein the mouse comprises a human immunoglobulin heavy chain variable region gene segment at an endogenous mouse immunoglobulin heavy chain variable region gene locus, the mouse lacks an endogenous functional ADAM6 sequence at the endogenous mouse immunoglobulin heavy chain variable region gene locus, and wherein the mouse comprises an ectopic nucleotide sequence that expresses a mouse ADAM6 protein or an ortholog or homolog or fragment thereof that is functional in a male mouse.

[0080] In one embodiment, the ectopic nucleotide sequence that expresses the mouse ADAM6 protein is extrachromosomal. In one embodiment, the ectopic nucleotide sequence that expresses the mouse ADAM6 protein is integrated at one or more loci in a genome of the mouse. In a specific embodiment, the one or more loci include an immunoglobulin locus.

[0081] In one aspect, a mouse is provided that expresses an immunoglobulin heavy chain sequence from a modified endogenous mouse immunoglobulin heavy chain locus, wherein the heavy chain is derived from a human V gene segment, a D gene segment, and a J gene segment, wherein the mouse comprises an ADAM6 activity that is functional in the mouse.

[0082] In one embodiment, the mouse comprises a single human V gene segment, a plurality of D gene segments, and a plurality of J gene segments. In one embodiment, the D gene segments are human D gene segments. In one embodiment, the J gene segments are human J gene segments. In one embodiment, the mouse further comprises a humanized heavy chain constant region sequence, wherein the humanization comprises replacement of a sequence selected from a C_H1, hinge, C_H2, C_H3, and a combination thereof. In a specific embodiment, the heavy chain is derived from the human V gene segment, a human D gene segment, a human J gene segment, a human C_H1 sequence, a human or mouse hinge sequence, a mouse C_H2 sequence, and a mouse C_H3 sequence. In another specific embodiment, the mouse further comprises a human light chain constant sequence.

[0083] In one embodiment, the mouse comprises an ADAM6 gene that is flanked 5' and 3' by endogenous immunoglobulin heavy chain gene segments. In a specific

embodiment, the endogenous immunoglobulin heavy chain gene segments are incapable of encoding a heavy chain of an antibody. In a specific embodiment, the ADAM6 gene of the mouse is at a position that is the same as in a wild-type mouse and the endogenous immunoglobulin heavy chain variable gene loci of the mouse are incapable of rearranging to encode a heavy chain of an antibody.

[0084] In one embodiment, the V gene segment is flanked 5' (with respect to transcriptional direction of the V gene segment) by a sequence encoding an ADAM6 activity that is functional in the mouse.

[0085] In one embodiment, the V gene segment is flanked 3' (with respect to transcriptional direction of the V gene segment) by a sequence encoding an ADAM6 activity that is functional in the mouse.

[0086] In one embodiment, the D gene segment is flanked 5' (with respect to transcriptional direction of the D gene segment) by a sequence encoding an ADAM6 activity that is functional in the mouse.

[0087] In one embodiment, the J gene segment is flanked 5' (with respect to transcriptional direction of the J gene segment) by a sequence encoding an ADAM6 activity that is functional in the mouse.

[0088] In one embodiment, the ADAM6 activity that is functional in the mouse results from expression of a nucleotide sequence located 5' of the 5'-most D gene segment and 3' of the single V gene segment (with respect to the direction of transcription of the V gene segment) of the modified endogenous mouse heavy chain immunoglobulin locus.

[0089] In one embodiment, the ADAM6 activity that is functional in the mouse results from expression of a nucleotide sequence located 5' of the 5'-most J gene segment and 3' of the 3'-most D gene segment (with respect to the direction of transcription of the D gene segment) of the modified endogenous mouse heavy chain immunoglobulin locus.

[0090] In one embodiment, the ADAM6 activity that is functional in the mouse results from expression of a nucleotide sequence located 5' of the single human V gene segment (with respect to the direction of transcription of the V gene segment) of the modified endogenous mouse heavy chain immunoglobulin locus.

[0091] In one embodiment, the nucleotide sequence comprises a sequence selected from a mouse ADAM6b sequence or functional fragment thereof, a mouse ADAM6a sequence or functional fragment thereof, and a combination thereof.

[0092] In one embodiment, the nucleotide sequence positioned upstream (5') or downstream (3') of the single human V gene segment is placed in opposite transcription orientation with respect to the human V gene segment. In a specific

embodiment, nucleotide sequence encodes, from 5' to 3' with respect to the direction of transcription of ADAM6 genes, and ADAM6a sequence followed by an ADAM6b sequence.

[0093] In one embodiment, the mouse comprises a single human V_H gene segment juxtaposed or contiguous with a mouse ADAM6 sequence or functional fragment thereof.

[0094] In one embodiment, the mouse comprises a human V_H1-69 gene segment juxtaposed or contiguous with a mouse ADAM6 sequence or functional fragment thereof.

[0095] In one embodiment, the mouse comprises a human V_H1-2 gene segment juxtaposed or contiguous with a mouse ADAM6 sequence or functional fragment thereof.

[0096] In one embodiment, the mouse comprises a single human V_H gene segment, and the mouse ADAM6 sequence or functional fragment thereof is juxtaposed or contiguous with endogenous immunoglobulin heavy chain gene segments, wherein the endogenous immunoglobulin heavy chain gene segments are incapable of rearranging to encode a heavy chain of an antibody.

[0097] In one embodiment, the sequence encoding the ADAM6 activity that is functional in the mouse is a mouse ADAM6 sequence or functional fragment thereof.

[0098] In one aspect, a mouse is provided that comprises a nucleic acid sequence encoding a mouse ADAM6 (or homolog or ortholog or functional fragment thereof) in a DNA-bearing cell of non-rearranged B cell lineage, but does not comprise the nucleic acid sequence encoding the mouse ADAM6 (or homolog or ortholog or functional fragment thereof) in a B cell that comprise rearranged immunoglobulin loci, wherein the nucleic acid sequence encoding the mouse ADAM6 (or homolog or ortholog or functional fragment thereof) occurs in the genome at a position that is different from a position in which a mouse ADAM6 gene appears in a wild-type mouse. In one embodiment, the nucleic acid sequence encoding the mouse ADAM6 (or homolog or ortholog or functional fragment thereof) is present in all or substantially all DNA-bearing cells that are not of rearranged B cell lineage; in one embodiment, the nucleic acid sequence is present in germline cells of the mouse, but not in a chromosome of a rearranged B cell.

[0099] In one aspect, a mouse is provided that comprises a nucleic acid sequence encoding a mouse ADAM6 (or homolog or ortholog or functional fragment thereof) in all or substantially all DNA-bearing cells, including B cells that comprise rearranged immunoglobulin loci, wherein the nucleic acid sequence encoding the mouse ADAM6 (or homolog or ortholog or functional fragment thereof) occurs in the

genome at a position that is different from a position in which a mouse ADAM6 gene appears in a wild-type mouse. In one embodiment, the nucleic acid sequence encoding the mouse ADAM6 (or homolog or ortholog or functional fragment thereof) is on a nucleic acid that is contiguous with the rearranged immunoglobulin locus. In one embodiment, the nucleic acid that is contiguous with the rearranged immunoglobulin locus is a chromosome. In one embodiment, the chromosome is a chromosome that is found in a wild-type mouse and the chromosome comprises a modification of a mouse immunoglobulin locus.

[00100] In one aspect, a genetically modified mouse is provided, wherein the mouse comprises a B cell that comprises in its genome an ADAM6 sequence or ortholog or homolog thereof. In one embodiment, the ADAM6 sequence or ortholog or homolog thereof is at an immunoglobulin heavy chain locus. In a specific embodiment, the heavy chain locus comprises endogenous immunoglobulin heavy chain gene segments that are incapable of rearranging to encode the heavy chain of an antibody. In one embodiment, the ADAM6 sequence or ortholog or homolog thereof is at a locus that is not an immunoglobulin locus. In one embodiment, the ADAM6 sequence is on a transgene driven by a heterologous promoter. In a specific embodiment, the heterologous promoter is a non-immunoglobulin promoter. In a specific embodiment, B cell expresses an ADAM6 protein or ortholog or homolog thereof.

[00101] In one embodiment, 90% or more of the B cells of the mouse comprise a gene encoding an ADAM6 protein or an ortholog thereof or a homolog thereof or a fragment thereof that is functional in the mouse. In a specific embodiment, the mouse is a male mouse.

[00102] In one embodiment, the B cell genome comprises a first allele and a second allele comprising the ADAM6 sequence or ortholog or homolog thereof. In one embodiment, the B cell genome comprises a first allele but not a second allele comprising the ADAM6 sequence or ortholog or homolog thereof.

[00103] In one aspect, a mouse is provided that comprises a modification at one or more endogenous immunoglobulin heavy chain alleles, wherein the modification maintains one or more endogenous ADAM6 alleles.

[00104] In one embodiment, the modification renders the mouse incapable of expressing a functional heavy chain that comprises rearranged endogenous heavy chain gene segments from at least one heavy chain allele and maintains an endogenous ADAM6 allele located within the at least one endogenous immunoglobulin heavy chain allele.

[00105] In one embodiment, the mice are incapable of expressing a functional

heavy chain that comprises rearranged endogenous heavy chain gene segments from at least one of the endogenous immunoglobulin heavy chain alleles, and the mice express and ADAM6 protein from an endogenous ADAM6 allele. In a specific embodiment, the mice are incapable of expressing a functional heavy chain that comprises rearranged endogenous heavy chain gene segments from two endogenous immunoglobulin heavy chain alleles, and the mice express an ADAM6 protein from one or more endogenous ADAM6 alleles.

[00106] In one embodiment, the mice are incapable of expressing a functional heavy chain from each endogenous heavy chain allele, and the mice comprise an functional ADAM6 allele located within 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, or 120 or more Mbp upstream (with respect to the direction of transcription of the mouse heavy chain locus) of a mouse immunoglobulin heavy chain constant region sequence. In a specific embodiment, the functional ADAM6 allele is at the endogenous immunoglobulin heavy chain locus (*e.g.*, in an intergenic V-D region, between two V gene segments, between a V and a D gene segment, between a D and a J gene segment, *etc.*). In a specific embodiment, the functional ADAM6 allele is located within a 90 to 100 kb intergenic sequence between the final mouse V gene segment and the first mouse D gene segment.

[00107] In one aspect, a mouse is provided that comprises a modification at one or more endogenous ADAM6 alleles.

[00108] In one embodiment, the modification renders the mouse incapable of expressing a functional ADAM6 protein from at least one of the one or more endogenous ADAM6 alleles. In a specific embodiment, the mouse is incapable of expressing a functional ADAM6 protein from each of the endogenous ADAM6 alleles.

[00109] In one embodiment, the mice are incapable of expressing a functional ADAM6 protein from each endogenous ADAM6 allele, and the mice comprise an ectopic ADAM6 sequence.

[00110] In one embodiment, the mice are incapable of expressing a functional ADAM6 protein from each endogenous ADAM6 allele, and the mice comprise an ectopic ADAM6 sequence located within 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, or 120 or more kb upstream (with respect to the direction of transcription of the mouse heavy chain locus) of a mouse immunoglobulin heavy chain constant region sequence. In a specific embodiment, the ectopic ADAM6 sequence is at the endogenous immunoglobulin heavy chain locus (*e.g.*, in an intergenic V-D region, between two V gene segments, between a V and a D gene segment, between a D and a J gene segment, *etc.*). In a specific embodiment, the ectopic ADAM6 sequence is located within a 90 to 100 kb intergenic sequence

between the final mouse V gene segment and the first mouse D gene segment. In another specific embodiment, the endogenous 90 to 100 kb intergenic V-D sequence is removed, and the ectopic ADAM6 sequence is placed between a single human V gene segment and a first human D gene segment. In another specific embodiment, the endogenous 90 to 100 kb intergenic V-D sequence is removed, and the ectopic ADAM6 sequence is placed 5' or upstream of the single human V gene segment.

[00111] In one aspect, an infertile male mouse is provided, wherein the mouse comprises a deletion of two or more endogenous ADAM6 alleles. In one aspect, a female mouse is provided that is a carrier of a male infertility trait, wherein the female mouse comprises in its germline a nonfunctional ADAM6 allele or a knockout of an endogenous ADAM6 allele.

[00112] In one aspect, a mouse comprising an endogenous immunoglobulin heavy chain V, D, and or J gene segment that are incapable of rearranging to encode an heavy chain of an antibody is provided, wherein the majority of the B cells of the mouse comprise an functional ADAM6 gene.

[00113] In one embodiment, the mouse comprises an intact endogenous immunoglobulin heavy chain V, D, and J gene segments that are incapable of rearranging to encode a functional heavy chain of an antibody. In one embodiment, the mouse comprises at least one and up to 89 V gene segments, at least one and up to 13 D gene segments, at least one and up to four J gene segments, and a combination thereof; wherein the at least one and up to 89 V gene segments, at least one and up to 13 D gene segments, at least one and up to four J gene segments are incapable of rearranging to encode a heavy chain variable region of an antibody. In a specific embodiment, the mouse comprises a functional ADAM6 gene located within the intact endogenous immunoglobulin heavy chain V, D, and J gene segments. In one embodiment, the mouse comprises an endogenous heavy chain locus that includes an endogenous ADAM6 locus, wherein the endogenous heavy chain locus comprises 89 V gene segments, 13 D gene segments, and four J gene segments, wherein the endogenous heavy chain gene segments are incapable of rearranging to encode a heavy chain variable region of an antibody and the ADAM6 locus encodes an ADAM6 protein that is functional in the mouse.

[00114] In one aspect, a mouse that lacks an endogenous immunoglobulin heavy chain V, D, and J gene segment is provided, wherein a majority of the B cells of the mouse comprise an ADAM6 sequence or ortholog or homolog thereof.

[00115] In one embodiment, the mouse lacks endogenous immunoglobulin heavy chain gene segments selected from two or more V gene segments, two or more D gene segments, two or more J gene segments, and a combination thereof. In one

embodiment, the mouse lacks immunoglobulin heavy chain gene segments selected from at least one and up to 89 V gene segments, at least one and up to 13 D gene segments, at least one and up to four J gene segments, and a combination thereof. In one embodiment, the mouse lacks a genomic DNA fragment from chromosome 12 comprising about three megabases of the endogenous immunoglobulin heavy chain locus. In a specific embodiment, the mouse lacks all functional endogenous heavy chain V, D, and J gene segments. In a specific embodiment, the mouse lacks 89 V_H gene segments, 13 D_H gene segments and four J_H gene segments.

[00116] In one aspect, a mouse is provided, wherein the mouse has a genome in the germline comprising a modification of an immunoglobulin heavy chain locus, wherein the modification to the immunoglobulin heavy chain locus comprises the replacement of one or more mouse immunoglobulin variable region sequences with one non-mouse immunoglobulin variable region sequences, and wherein the mouse comprises a nucleic acid sequence encoding a mouse ADAM6 protein. In a preferred embodiment, the D_H and J_H sequences and at least 3, at least 10, at least 20, at least 40, at least 60, or at least 80 V_H sequences of the endogenous immunoglobulin heavy chain locus are replaced by non-mouse immunoglobulin heavy chain sequences. In a further preferred embodiment, the D_H, J_H, and all V_H sequences of the endogenous immunoglobulin heavy chain locus are replaced by a single non-mouse immunoglobulin V gene segment, one or more D gene segment, and one or more J gene segment sequences. The non-mouse immunoglobulin sequences can be unrearranged. In a preferred embodiment, the non-mouse immunoglobulin sequences comprise complete unrearranged D_H and J_H regions and a single unrearranged V_H sequence of the non-mouse species. In a further preferred embodiment, the non-mouse immunoglobulin sequences are capable of forming a complete variable region, *i.e.*, a rearranged variable region containing V_H, D_H, and J_H segments joined together to form a sequence that encodes a heavy chain variable region, of the non-mouse species. The non-mouse species can be Homo sapiens and the non-mouse immunoglobulin sequences can be human sequences.

[00117] In one aspect, a heavy chain immunoglobulin locus is provided that comprises a single functional human V segment. In one embodiment, the single functional human V segment is selected from a V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23, V_H3-30, V_H3-30-3, V_H3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, and a V_H7-81 segment. In one embodiment, the

single functional human V segment is a V_H1-69 segment; in a specific embodiment, the single functional human V segment is present in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 polymorphic forms found in the human population. In one embodiment, the single functional human V segment is a V_H1-2 segment; in a specific embodiment, the single functional human V segment is present in 1, 2, 3, 4, or 5 polymorphic forms found in the human population.

[00118] In one embodiment, the heavy chain immunoglobulin locus is a modified locus of a non-human animal. In one embodiment, the modified non-human immunoglobulin heavy chain locus is present in the non-human animal at a position in the genome in which the corresponding unmodified non-human locus is found in the wild-type non-human animal. In one embodiment, the modified non-human immunoglobulin heavy chain locus is present on a transgene in a non-human animal.

[00119] In one embodiment, the single functional human V gene segment is a V_H1-69 gene segment. In one embodiment, the V_H1-69 gene segment comprises SEQ ID NO: 37. In one embodiment, the V_H1-69 gene segment is derived from SEQ ID NO: 37. In one embodiment, the V_H1-69 gene segment is at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to SEQ ID NO: 37.

[00120] In one embodiment, the single functional human V gene segment is encoded by the nucleotide sequence of SEQ ID NO: 37.

[00121] In one embodiment, the single functional human V gene segment is a V_H1-2 gene segment. In one embodiment, the V_H1-2 gene segment comprises SEQ ID NO: 63. In one embodiment, the V_H1-2 gene segment is derived from SEQ ID NO: 63. In one embodiment, the V_H1-2 gene segment is at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to SEQ ID NO: 63.

[00122] In one embodiment, the single functional human V gene segment is encoded by a nucleotide sequence comprising SEQ ID NO: 63.

[00123] In one embodiment, the single functional human V segment is operably linked to one or more D segments and one or more J segments, or one or more J segments. In one embodiment, the V segment and one or more D and/or J segments are operably linked to an immunoglobulin heavy chain constant region sequence. In one embodiment the immunoglobulin heavy chain constant region sequence is selected from a C_H1, a hinge, a C_H2, a C_H3 sequence, and a combination thereof. In one embodiment, the C_H1, hinge, C_H2, C_H3, or combination thereof are each non-human endogenous constant sequences. In one embodiment, at least one of the C_H1, hinge, C_H2, C_H3, or combination thereof is a human sequence. In a specific embodiment, the C_H1 and/or hinge are human sequences.

[00124] In one aspect, a modified endogenous non-human immunoglobulin heavy chain locus is provided, comprising a replacement of all functional V segments with a single human V segment, wherein the non-human immunoglobulin heavy chain locus is incapable of rearrangement to form a heavy chain variable gene that is derived from a V segment other than the single human V segment.

[00125] In one embodiment, the single human V segment is V_H1-69. In one embodiment, the single human V segment is V_H1-2.

[00126] In one embodiment, the locus comprises at least one human or non-human D_H segment, and one human or non-human J_H segment. In a specific embodiment, the locus comprises a human D_H segment and a human J_H segment. In a specific embodiment, the locus comprises a human J_H segment. In another specific embodiment, the locus comprises a human V_H1-69, all functional human D_H segments, and all functional human J_H segments. In one embodiment, the human V, D, and J segments (or V and J segments) are operably linked to a mouse constant region gene at an endogenous mouse heavy chain locus. In a specific embodiment, the mouse heavy chain locus comprises a wild-type repertoire of mouse immunoglobulin constant region sequences.

[00127] In one aspect, a genetically modified non-human animal is provided, wherein the only functional immunoglobulin heavy chain V gene segment of the non-human animal is selected from a human V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23, V_H3-30, V_H3-30-3, V_H3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, and a V_H7-81 segment. In one embodiment, the heavy chain V gene segment is a human V_H1-69 gene segment. In one embodiment, the heavy chain V gene segment is a human V_H1-2 gene segment.

[00128] In one aspect, a genetically modified non-human animal is provided, wherein the non-human animal comprises a single functional human V_H segment, and wherein the non-human animal is substantially incapable of forming a rearranged immunoglobulin heavy chain variable domain gene that lacks the single functional human V_H segment.

[00129] In one aspect, a genetically modified non-human animal is provided, wherein the only immunoglobulin heavy chain variable region expressed in the non-human animal is derived from one of a human segment selected from a human V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23,

V_H3-30, V_H3-30-3, V_H3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, and a V_H7-81 gene segment. In one embodiment, the human segment is a V_H1-69 segment. In one embodiment, the human segment is a V_H1-2 segment. In one embodiment, the only immunoglobulin heavy chain variable region expressed by the mouse is derived from a single V segment family member, and in one embodiment the only immunoglobulin heavy chain variable region is derived from a polymorphic variant of the single V segment family member.

[00130] In one aspect, a non-human animal comprising a restricted immunoglobulin heavy chain V gene segment repertoire is provided, wherein the non-human animal further comprises one or more human immunoglobulin κ light chain variable segments (Vκ). In one embodiment, the one or more Vκ segments are operably linked to one or more human J segments. In a specific embodiment, the J segments are human Jκ segments. In another specific embodiment, the non-human animal does not express an immunoglobulin λ light chain. In another specific embodiment, the non-human animal does not comprise a functional human or functional endogenous immunoglobulin λ light chain variable locus.

[00131] In one embodiment, the non-human animal is a mouse.

[00132] In one embodiment, the non-human animal comprises a replacement at the endogenous non-human immunoglobulin Vκ locus of all or substantially all functional endogenous Vκ segments with one or more functional human Vκ segments. In a further specific embodiment, the replacement is with all or substantially all functional human immunoglobulin Vκ segments.

[00133] In one embodiment, the non-human animal comprises a replacement at the endogenous non-human immunoglobulin Jκ locus of all or substantially all functional endogenous non-human immunoglobulin Jκ segments with one or more functional human immunoglobulin Jκ segments. In a further specific embodiment, the replacement is with all or substantially all functional human immunoglobulin Jκ segments.

[00134] In a specific embodiment, the non-human animal comprises an immunoglobulin heavy chain variable region locus that comprises a repertoire of V segments consisting essentially of a single V segment and/or polymorphic variants thereof. In one embodiment, the single immunoglobulin heavy chain V segment is a human V_H1-69 segment, and the non-human animal further comprises a replacement of all functional non-human D_H segments with all functional human D_H segments, and

further comprises a replacement of all functional non-human J_H segments with all functional human J_H segments, and wherein the immunoglobulin heavy chain variable region locus is operably linked to a human or non-human constant region gene sequence. In a specific embodiment, the constant region gene sequence is an endogenous non-human constant region gene sequence. In a specific embodiment, the non-human animal rearranges segments at the non-human immunoglobulin heavy chain locus to form a gene encoding heavy chain variable region comprising a human V_H1-69 sequence, a human D_H sequence, a human J_H sequence, and a mouse constant region sequence.

[00135] In a specific embodiment, the non-human animal comprises an immunoglobulin heavy chain variable region locus that comprises a repertoire of V segments consisting essentially of a single V segment and/or polymorphic variants thereof. In one embodiment, the single immunoglobulin heavy chain V segment is a human V_H1-2 segment, and the non-human animal further comprises a replacement of all functional non-human D_H segments with all functional human D_H segments, and further comprises a replacement of all functional non-human J_H segments with all functional human J_H segments, and wherein the immunoglobulin heavy chain variable region locus is operably linked to a human or non-human constant region gene sequence. In a specific embodiment, the constant region gene sequence is an endogenous non-human constant region gene sequence. In a specific embodiment, the non-human animal rearranges segments at the non-human immunoglobulin heavy chain locus to form a gene encoding heavy chain variable region comprising a human V_H1-2 sequence, a human D_H sequence, a human J_H sequence, and a mouse constant region sequence.

[00136] In one embodiment, a B cell is provided that comprises the rearranged gene. In a specific embodiment, the B cell is from a mouse as described that has been immunized with an antigen of interest, and the B cell encodes an antibody that specifically binds the antigen of interest. In one embodiment, the antigen of interest is a pathogen. In a specific embodiment, the pathogen is selected from an influenza virus, a hepatitis virus (*e.g.*, hepatitis B or hepatitis C virus), and a human immunodeficiency virus. In a specific embodiment, the B cell encodes a somatically mutated, high affinity (*e.g.*, about 10^{-9} K_D or lower) antibody comprising a human light chain variable region (*e.g.*, a human κ light chain variable region) that specifically binds the antigen of interest.

[00137] In one aspect, a non-human animal comprising a restricted immunoglobulin heavy chain V segment repertoire is provided, wherein the non-human animal comprises one or more human λ light chain variable (Vλ) segments.

In one embodiment, the one or more human Vλ segments are operably linked to one or more human J segments. In a specific embodiment, the J segments are human Jλ segments. In another specific embodiment, the non-human animal does not express a κ light chain. In another specific embodiment, the non-human animal does not comprise a functional human or non-human κ light chain variable locus.

[00138] In one embodiment, the non-human animal comprises a replacement of all or substantially all functional non-human immunoglobulin Vλ segments with one or more functional human immunoglobulin Vλ segments. In a further specific embodiment, the replacement is with all or substantially all functional human immunoglobulin Vλ segments.

[00139] In one embodiment, the non-human animal comprises a replacement of all or substantially all functional non-human immunoglobulin Jλ segments with one or more functional human immunoglobulin Jλ segments. In a further specific embodiment, the replacement is with all or substantially all functional human immunoglobulin Jλ segments.

[00140] In a specific embodiment, the non-human animal comprises an immunoglobulin heavy chain variable (V_H) region locus that comprises only a single V_H segment, wherein the single V_H segment is a human V_H1-69 segment or a human V_H1-2 segment, and further comprises a replacement of all functional non-human D_H segments with all functional human D_H segments, and further comprises a replacement of all functional non-human J_H segments with all functional human J_H segments, and wherein the V_H region locus is operably linked to a human or non-human constant region gene sequence. In a specific embodiment, the constant region gene sequence is a non-human constant region gene sequence, *e.g.,* an endogenous non-human constant gene sequence. In a specific embodiment, the non-human animal rearranges segments at the non-human immunoglobulin heavy chain locus to form a gene encoding an immunoglobulin heavy chain variable region comprising a human V_H1-69 sequence (or a human V_H1-2 sequence), a human D_H sequence, a human J_H sequence, and an endogenous non-human constant region sequence.

[00141] In one embodiment, a B cell is provided that comprises the rearranged gene. In a specific embodiment, the B cell is from a non-human animal as described that has been immunized with an antigen of interest, and the B cell encodes an antibody that specifically binds the antigen of interest. In one embodiment, the antigen is a human protein selected from a ligand, a cell surface receptor and an intracellular protein. In one embodiment, the antigen of interest is a pathogen. In a

specific embodiment, the pathogen is selected from an influenza virus, a hepatitis virus (*e.g.*, hepatitis B or hepatitis C virus), and a human immunodeficiency virus. In a specific embodiment, the B cell encodes a somatically mutated, high affinity (*e.g.*, about 10^{-9} K_D or lower) antibody comprising a human light chain variable region (*e.g.*, a human λ light chain variable region) that specifically binds the antigen of interest.

[00142] In one aspect, a non-human animal comprising a restricted immunoglobulin heavy chain V segment repertoire is provided, wherein the non-human animal comprises a human V_H1-69 segment (or a human V_H1-2 segment) on a transgene, wherein the human V_H1-69 segment is operably linked on the transgene to a human or non-human D_H segment, and/or a human or non-human J segment, and the transgene further comprises a human or non-human constant region gene, or a chimeric human/non-human constant region (*e.g.*, a C_H1, hinge, C_H2, C_H3 or combination thereof wherein at least one sequence is non-human, *e.g.*, selected from hinge, C_H2, and C_H3 and/or hinge). In one embodiment, the non-human animal is a mouse or rat and the non-human D, J, and/or constant region gene is a mouse or rat gene or chimeric human/mouse or rat.

[00143] In one embodiment, the non-human animal comprises a transgene that comprises an immunoglobulin light chain variable region locus that comprises one or more human immunoglobulin Vλ segments and Jλ segments, or one or more human immunoglobulin Vκ segments and Jκ segments, and a human immunoglobulin κ or λ light chain constant region gene, such that the transgene rearranges in the non-human animal to form a rearranged immunoglobulin κ or λ light chain gene.

[00144] In a specific embodiment, the non-human animal comprises a transgene having an immunoglobulin heavy chain variable locus that comprises a single V segment that is a human V_H1-69 segment (or a human V_H1-2 segment), one or more human D segments, one or more human J segments, and a human constant gene operably linked to the heavy chain variable locus, such that the mouse expresses from the transgene a fully human antibody derived from the V_H1-69 segment (or the V_H1-2 segment). In one embodiment, the non-human animal does not comprise a functional endogenous immunoglobulin heavy chain variable region locus. In a specific embodiment, the non-human animal comprises a nonfunctional endogenous immunoglobulin heavy chain variable region locus that comprises a deletion of an endogenous non-human D_H and/or endogenous non-human J_H segment, such that the non-human animal is incapable of rearranging the endogenous immunoglobulin heavy chain variable region locus to form a rearranged non-human antibody gene. In a specific embodiment, the non-human animal comprises a deletion of a switch

sequence operably linked to an endogenous mouse heavy chain constant region. In a specific embodiment, the switch sequence is a non-human (*e.g.*, mouse) μ switch sequence. In another embodiment, the non-human animal further comprises a lack of a functional endogenous light chain variable locus selected from an immunoglobulin κ locus and an immunoglobulin λ locus. In a specific embodiment, the non-human animal comprises a deletion of a Jκ and/or a Jλ sequence, such that the non-human animal is incapable of rearranging an endogenous non-human immunoglobulin κ light chain and/or an endogenous non-human immunoglobulin λ light chain variable region to form a rearranged endogenous non-human immunoglobulin κ light chain and/or a rearranged endogenous non-human immunoglobulin λ light chain gene.

[00145] In one embodiment, the non-human animal comprises a deletion of an endogenous non-human immunoglobulin κ light chain sequence that results in a functional knockout of the endogenous non-human immunoglobulin κ light chain. In one embodiment, the non-human animal comprises a deletion of an endogenous non-human immunoglobulin λ light chain sequence that results in a functional knockout of the endogenous non-human immunoglobulin λ light chain.

[00146] In one aspect, a rodent is provided that comprises an immunoglobulin heavy chain variable repertoire derived from no more than one human V_H segment or one or more polymorphs thereof, from a D segment selected from a repertoire of one or more D segments, and from a J segment selected from a repertoire of one or more J segments; wherein the rodent comprises an ectopic ADAM6 sequence or ortholog or homolog or fragment thereof that is functional in a male rodent.

[00147] In one embodiment, the human V_H segment is present in 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more polymorphic variants, wherein each polymorphic variant is operably linked to a D and/or J segment such that each polymorphic variant is capable of rearranging and forming a rearranged heavy chain variable domain with any of the one or more D segments and any of the one or more J segments. In one embodiment, the rodent is a mouse or a rat. In one embodiment, the repertoire of D segments comprises two or more D segments. In one embodiment, the repertoire of J segments comprises two or more J segments. In one embodiment, the D and/or J segments are human segments. In one embodiment, the ectopic ADAM6 sequence is an ADAM6 sequence of a wild-type rodent of the same species. In one embodiment, the rodent is a mouse or a rat. In one embodiment, the ectopic ADAM6 sequence or ortholog or homolog or fragment thereof that is functional in the male

rodent is on the same chromosome as the modified immunoglobulin heavy chain variable repertoire; in one embodiment, it is on a different chromosome.

[00148] In one aspect, a nucleotide construct is provided that comprises a sequence encoding a single human immunoglobulin heavy chain V_H segment and/or polymorphic variants thereof and one or more D_H and one or more J sequences, wherein the construct comprises at least one homology arm homologous to a non-human immunoglobulin heavy chain variable locus, or a recombinase recognition site (*e.g.*, a lox site). In one embodiment, the V segment is a V_H1-69 segment or a V_H1-2 segment.

[00149] In one aspect, a nucleotide construct is provided, comprising a nucleotide sequence encoding a single human immunoglobulin heavy chain V segment, wherein the single V_H segment is a V_H1-69 (or V_H1-2) segment. In one embodiment, the construct comprises a site-specific recombinase recognition site. In one embodiment, the construct comprises a first mouse homology arm upstream of the V_H1-69 (or V_H1-2) segment and a second mouse homology arm downstream of the V_H1-69 (or V_H1-2) segment, and wherein the first mouse homology arm is homologous to a region of a mouse chromosome immediately upstream of a mouse immunoglobulin heavy chain variable region but not including a functional mouse immunoglobulin heavy chain variable segment. In one embodiment, the construct comprises SEQ ID NO: 6. In one embodiment, the construct comprises SEQ ID NO: 74. In one embodiment, the construct comprises SEQ ID NO: 75. In one embodiment, the construct comprises SEQ ID NO: 76.

[00150] In one aspect, the restricted single V_H segment is in a non-human animal, or the restricted V_H segment is at a non-human immunoglobulin heavy chain locus (*e.g., in situ* or in a transgene), and the non-human animal or non-human immunoglobulin heavy chain locus is selected from a mouse, rat, rabbit, pig, bovine (*e.g.*, cow, bull, buffalo), deer, sheep, goat, chicken, cat, dog, ferret, primate (*e.g.*, marmoset, rhesus monkey) locus or animal. In a specific embodiment, the non-human animal or locus is a mouse or a rat locus.

[00151] In one aspect, a targeting vector is provided, comprising (a) a nucleotide sequence that is identical or substantially identical to a human variable region gene segment nucleotide sequence; and, (b) a nucleotide sequence encoding a mouse ADAM6 or ortholog or homolog or fragment thereof that is functional in a mouse.

[00152] In one embodiment, the targeting vector further comprises a promoter operably linked to the sequence encoding the mouse ADAM6. In a specific embodiment, the promoter is a mouse ADAM6 promoter.

[00153] In one aspect, a nucleotide construct for modifying a mouse

immunoglobulin heavy chain variable locus is provided, wherein the construct comprises at least one site-specific recombinase recognition site and a sequence encoding an ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a mouse.

[00154] In one aspect, a nucleic acid construct is provided, comprising an upstream homology arm and a downstream homology arm, wherein the upstream homology arm comprises a sequence that is identical or substantially identical to a human immunoglobulin heavy chain variable region sequence, the downstream homology arm comprises a sequence that is identical or substantially identical to a human or mouse immunoglobulin variable region sequence, and disposed between the upstream and downstream homology arms is a sequence that comprises a nucleotide sequence encoding a mouse ADAM6 protein. In a specific embodiment, the sequence encoding the mouse ADAM6 gene is operably linked with a mouse promoter with which the mouse ADAM6 is linked in a wild type mouse.

[00155] In one aspect, a cell isolated from a genetically modified mouse as described herein is provided. In one embodiment, the cell is a lymphocyte. In one embodiment, the lymphocyte is a B cell. In a specific embodiment, the B cell comprises an ectopic ADAM6 sequence or ortholog or homolog or sequence encoding a functional fragment thereof, wherein the B cell expresses a heavy chain variable domain derived from a human V_H gene segment.

[00156] In one aspect, a cell or tissue is provided, wherein the cell or tissue is derived from a non-human animal as described herein, and comprises a restricted V_H segment repertoire. In one embodiment, the V_H segment repertoire is restricted to a single V_H segment family member and/or polymorphic variants thereof. In a specific embodiment, the single V_H segment is a human V_H1-69 segment or a human V_H1-2 segment. In one embodiment, the cell or tissue is derived from spleen, lymph node or bone marrow of the non-human animal.

[00157] In one embodiment, the cell is an ES cell. In one embodiment, the cell is a B cell. In one embodiment, the cell is a germ cell.

[00158] In one embodiment, the tissue is selected from connective, muscle, nervous and epithelial tissue. In a specific embodiment, the tissue is reproductive tissue.

[00159] In one embodiment, the cell and/or tissue derived from a mouse as described herein is isolated for use in one or more *ex vivo* assays. In various embodiments, the one or more *ex vivo* assays include measurements of physical, thermal, electrical, mechanical or optical properties, a surgical procedure,

measurements of interactions of different tissue types, the development of imaging techniques, or a combination thereof.

[00160] In one embodiment, the non-human animal is a mouse.

[00161] In one aspect, a non-human embryo is provided comprising a restricted heavy chain V_H segments as described herein. In one embodiment, the embryo comprises an ES donor cell that comprises the restricted V_H segment, and host embryo cells.

[00162] In one embodiment, the non-human animal is a mouse.

[00163] In one aspect, a non-human cell comprising a chromosome or fragment thereof of a non-human animal as described herein. In one embodiment, the non-human cell comprises a nucleus of a non-human animal as described herein. In one embodiment, the non-human cell comprises the chromosome or fragment thereof as the result of a nuclear transfer.

[00164] 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.

[00165] In one aspect, a pluripotent, induced pluripotent, or totipotent cell derived from a non-human animal as described herein is provided. In a specific embodiment, the cell is a mouse embryonic stem (ES) cell.

[00166] In one aspect, a non-human induced pluripotent cell comprising a restricted V_H segment repertoire is provided. In one embodiment, the induced pluripotent cell is derived from a non-human animal as described herein.

[00167] In one aspect a hybridoma is provided, comprising a sequence of a lymphocyte of a mouse as described herein. In one embodiment, the lymphocyte is a B cell.

[00168] In one aspect, a hybridoma or quadroma is provided, derived from a cell of a non-human animal as described herein. In one embodiment, the non-human animal is a mouse or rat.

[00169] In one aspect, mouse cells and mouse embryos are provided, including but not limited to ES cells, pluripotent cells, and induced pluripotent cells, that comprise genetic modifications as described herein. Cells that are XX and cells that are XY are provided. Cells that comprise a nucleus containing a modification as described herein are also provided, *e.g.*, a modification introduced into a cell by pronuclear injection. Cells, embryos, and mice that comprise a virally introduced ADAM6 gene are also provided, *e.g.*, cells, embryos, and mice comprising a transduction construct comprising an ADAM6 gene that is functional in the mouse.

[00170] In one aspect, a genetically modified mouse cell is provided, wherein the

cell is incapable of expressing a heavy chain comprising rearranged endogenous immunoglobulin heavy chain gene segments, and the cell comprises a functional ADAM6 gene that encodes a mouse ADAM6 protein or functional fragment thereof. In one embodiment, the cell further comprises an insertion of human immunoglobulin gene segments. In a specific embodiment, the human immunoglobulin gene segments are heavy chain gene segments that are operably linked to mouse heavy chain constant regions such that upon rearrangement encode a functional heavy chain of an antibody that comprises a human variable region.

[00171] In one aspect, a genetically modified mouse cell is provided; wherein the cell lacks a functional endogenous mouse ADAM6 locus, and the cell comprises an ectopic nucleotide sequence that encodes a mouse ADAM6 protein or functional fragment thereof. In one embodiment, the cell further comprises a modification of an endogenous immunoglobulin heavy chain variable gene sequence. In a specific embodiment, the modification of the endogenous immunoglobulin heavy chain variable gene sequence comprises a deletion selected from a deletion of a mouse V_H gene segment, a deletion of a mouse D_H gene segment, a deletion of a mouse J_H gene segment, and a combination thereof. In a specific embodiment, the mouse comprises a replacement of one or more mouse immunoglobulin V_H, D_H, and/or J_H sequences with a human immunoglobulin sequence. In a specific embodiment, the human immunoglobulin sequence is selected from a human V_H, a human V_L, a human D_H, a human J_H, a human J_L, and a combination thereof.

[00172] In one embodiment, the cell is a totipotent cell, a pluripotent cell, or an induced pluripotent cell. In a specific embodiment, the cell is a mouse ES cell.

[00173] In one aspect, a mouse B cell is provided, wherein the mouse B cell comprises a rearranged immunoglobulin heavy chain gene, wherein the B cell comprises on a chromosome of the B cell a nucleic acid sequence encoding an ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a male mouse. In one embodiment, the mouse B cell comprises two alleles of the nucleic acid sequence.

[00174] In one embodiment, the nucleic acid sequence is on a nucleic acid molecule (*e.g.*, a B cell chromosome) that is contiguous with the rearranged mouse immunoglobulin heavy chain locus.

[00175] In one embodiment, the nucleic acid sequence is on a nucleic acid molecule (*e.g.*, a B cell chromosome) that is distinct from the nucleic acid molecule that comprises the rearranged mouse immunoglobulin heavy chain locus.

[00176] In one embodiment, the mouse B cell comprises a rearranged non-mouse immunoglobulin variable gene sequence operably linked to a mouse or human

immunoglobulin constant region gene, wherein the B cell comprises a nucleic acid sequence that encodes an ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a male mouse.

[00177] In one embodiment, the nucleic acid sequence is on a nucleic acid molecule (*e.g.*, a B cell chromosome) that is located at or within the nearest gene locus with respect to the rearranged non-human immunoglobulin variable gene sequence.

[00178] In one embodiment, the nucleic acid sequence is on a nucleic acid molecule (*e.g.*, a B cell chromosome) that is contiguous with the rearranged non-human immunoglobulin variable region sequence.

[00179] In one aspect, a somatic mouse cell is provided, comprising a chromosome that comprises a modified immunoglobulin heavy chain locus, and a nucleic acid sequence encoding a mouse ADAM6 or ortholog or homolog or fragment thereof that is functional in a male mouse. In one embodiment, the nucleic acid sequence is on the same chromosome as the modified immunoglobulin heavy chain locus. In one embodiment, the nucleic acid is on a different chromosome than the modified immunoglobulin heavy chain locus. In one embodiment, the somatic cell comprises a single copy of the nucleic acid sequence. In one embodiment, the somatic cell comprises at least two copies of the nucleic acid sequence. In a specific embodiment, the somatic cell is a B cell. In a specific embodiment, the cell is a germ cell. In a specific embodiment, the cell is a stem cell.

[00180] In one aspect, a mouse germ cell is provided, comprising a nucleic acid sequence encoding a mouse ADAM6 (or homolog or ortholog or functional fragment thereof) on a chromosome of the germ cell, wherein the nucleic acid sequence encoding the mouse ADAM6 (or homolog or ortholog or functional fragment thereof) is at a position in the chromosome that is different from a position in a chromosome of a wild-type mouse germ cell. In one embodiment, the nucleic acid sequence is at a mouse immunoglobulin locus. In one embodiment, the nucleic acid sequence is on the same chromosome of the germ cell as a mouse immunoglobulin locus. In one embodiment, the nucleic acid sequence is on a different chromosome of the germ cell than the mouse immunoglobulin locus. In one embodiment, the mouse immunoglobulin locus comprises a replacement of at least one mouse immunoglobulin sequence with at least one non-mouse immunoglobulin sequence. In a specific embodiment, the at least one non-mouse immunoglobulin sequence is a human immunoglobulin sequence. In one embodiment, the human immunoglobulin sequence is an immunoglobulin heavy chain sequence.

[00181] In one aspect, an antibody variable domain sequence made in a non-human animal as described herein is provided.

[00182] In one aspect, a human therapeutic is provided, comprising an antibody variable domain comprising a sequence derived from a non-human animal as described herein.

[00183] In one aspect, a method of obtaining an antibody variable region sequence from a non-human animal is provided, wherein the antibody variable region sequence is derived from a human V_H1-69 segment or a V_H1-2 segment, wherein the method comprises (a) immunizing a non-human animal with an antigen of interest, wherein the non-human animal comprises a replacement at the endogenous immunoglobulin heavy chain locus of all or substantially all non-human variable segments with a single human variable segment, wherein the single human variable segment is a V_H1-69 segment or a V_H1-2 segment, and wherein the non-human animal is substantially incapable of forming a immunoglobulin heavy chain variable region sequence that is not derived from a human V_H1-69 segment or a V_H1-2 segment; (b) allowing the non-human animal to mount an immune response with respect to the antigen of interest; and, (c) identifying or isolating an immunoglobulin heavy chain variable region sequence of the non-human animal, wherein the antibody binds the antigen of interest.

[00184] In one embodiment, the single human variable segment is a V_H1-69 segment.

[00185] In one embodiment, the antibody variable region sequence is derived from SEQ ID NO: 37. In one embodiment, the antibody variable region sequence is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to SEQ ID NO: 37. In one embodiment, the antibody variable region sequence comprises SEQ ID NO: 37.

[00186] In one embodiment the single human variable segment is a V_H1-2 segment.

[00187] In one embodiment, the antibody variable region sequence is derived from SEQ ID NO: 63. In one embodiment, the antibody variable region sequence is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to SEQ ID NO: 63. In one embodiment, the antibody variable region sequence comprises SEQ ID NO: 63.

[00188] In one aspect, a method for generating a repertoire of human antibody variable regions in a non-human animal is provided, wherein the human heavy chain variable regions of the repertoire are derived from the same V_H gene family member and one of a plurality of D_H segments and one of a plurality of J_H segments, wherein

the repertoire is characterized by having heavy chain immunoglobulin FR1 (framework 1), CDR1, FR2, CDR2, and FR3 sequences from a single V_H gene family member. In one embodiment, the repertoire is further characterized by having a plurality of different CDR3 + FR4 sequences.

[00189] In one embodiment, the single V_H gene family is selected from V_H family 1, 2, 3, 4, 5, 6, and 7. In a specific embodiment, the single V_H gene family is V_H family 1. In one embodiment, the single V_H gene family member is selected from V_H1-2, V_H1-69, V_H2-26, V_H2-70, and V_H3-23. In a specific embodiment, the single V_H gene family member is V_H1-69.

[00190] In one embodiment, the repertoire comprises heavy chain FR1, CDR1, FR2, CDR2 and FR3 sequences derived from a V_H1-69 segment. In a specific embodiment, the repertoire comprises heavy chain FR1, CDR1, FR2, CDR2 and FR3 sequences derived from SEQ ID NO: 38. In a specific embodiment, the repertoire comprises heavy chain FR1, CDR1, FR2, CDR2 and FR3 sequences of SEQ ID NO: 38.

[00191] In one embodiment, the repertoire comprises heavy chain FR1, CDR1, FR2, CDR2 and FR3 sequences derived from a V_H1-2 segment. In a specific embodiment, the repertoire comprises heavy chain FR1, CDR1, FR2, CDR2 and FR3 sequences derived from SEQ ID NO: 64. In a specific embodiment, the repertoire comprises heavy chain FR1, CDR1, FR2, CDR2 and FR3 sequences of SEQ ID NO: 64.

[00192] In one aspect, a method for generating a plurality of different CDR3 and FR4 sequences in a non-human animal is provided, comprising exposing a non-human animal that comprises an immunoglobulin heavy chain variable gene locus with a V_H segment repertoire restricted to a single V_H segment family member to an antigen of interest, allowing the non-human animal to develop an immune response to the antigen, wherein the immune response generates a B cell repertoire whose heavy chain variable domains are each derived from the single V_H segment family member and that comprise a plurality of different CDR3 and FR4 sequences.

[00193] In one embodiment, the singe V_H segment family member is human. In one embodiment, the non-human animal is selected from a mouse, a rat, and a rabbit. In one embodiment, the antigen of interest is selected from a ligand, a receptor, an intracellular protein and a secreted protein. In one embodiment, the antigen of interest is a human pathogen.

[00194] In one aspect, a nucleotide sequence encoding an immunoglobulin variable region made in a non-human animal as described herein is provided.

[00195] In one aspect, an immunoglobulin heavy chain or immunoglobulin light

chain variable region amino acid sequence of an antibody made in a non-human animal as described herein is provided.

[00196] In one aspect, an immunoglobulin heavy chain or immunoglobulin light chain variable region nucleotide sequence encoding a variable region of an antibody made in a non-human as described herein is provided.

[00197] In one aspect, an antibody or antigen-binding fragment thereof (*e.g.*, Fab, $F(ab)_2$, scFv) made in a non-human animal as described herein is provided.

[00198] In one aspect, a method for making a genetically modified non-human animal is provided, comprising replacing one or more immunoglobulin heavy chain gene segments upstream (with respect to transcription of the immunoglobulin heavy chain gene segments) of an endogenous ADAM6 locus of the non-human animal with one or more human immunoglobulin heavy chain gene segments, and replacing one or more immunoglobulin gene segments downstream (with respect to transcription of the immunoglobulin heavy chain gene segments) of the ADAM6 locus of the non-human animal with one or more human immunoglobulin heavy chain or light chain gene segments. In one embodiment, the one or more human immunoglobulin gene segments replacing one or more endogenous immunoglobulin gene segments upstream of an endogenous ADAM6 locus of the non-human animal include V gene segments. In one embodiment, the human immunoglobulin gene segments replacing one or more endogenous immunoglobulin gene segments upstream of an endogenous ADAM6 locus of the non-human animal include V and D gene segments. In one embodiment, the one or more human immunoglobulin gene segments replacing one or more endogenous immunoglobulin gene segments downstream of an endogenous ADAM6 locus of the non-human animal include J gene segments. In one embodiment, the one or more human immunoglobulin gene segments replacing one or more endogenous immunoglobulin gene segments downstream of an endogenous ADAM6 locus of the non-human animal include D and J gene segments. In one embodiment, the one or more human immunoglobulin gene segments replacing one or more endogenous immunoglobulin gene segments downstream of an endogenous ADAM6 locus of the non-human animal include V, D and J gene segments. In a specific embodiment, the one or more gene segments replacing one or more endogenous immunoglobulin gene segments downstream of an endogenous ADAM6 locus of the non-human animal includes a single V gene segment, one or more D gene segments and one or more J gene segments.

[00199] In one embodiment, the one or more immunoglobulin heavy chain gene segments upstream and/or downstream of the ADAM6 gene are replaced in a pluripotent, induced pluripotent, or totipotent cell to form a genetically modified

progenitor cell; the genetically modified progenitor cell is introduced into a host; and, the host comprising the genetically modified progenitor cell is gestated to form a non-human animal comprising a genome derived from the genetically modified progenitor cell. In one embodiment, the host is an embryo. In a specific embodiment, the host is selected from a mouse pre-morula (*e.g.,* 8- or 4-cell stage), a tetraploid embryo, an aggregate of embryonic cells, or a blastocyst.

[00200] In one aspect, a non-human animal is provided, wherein the non-human animal has a B cell repertoire that expresses immunoglobulin heavy chain variable domains derived from a single V segment family member. In one embodiment, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90, or at least 95% of the B cell repertoire of the non-human animal immunoglobulin heavy chain variable domain expressed in the B cell repertoire is derived from the same V segment family member. In a specific embodiment, the percentage is at least 90%. In one embodiment, the B cell repertoire consists essentially of peripheral (blood) B cells. In one embodiment, the B cell repertoire consists essentially of splenic B cells. In one embodiment, the B cell repertoire consists essentially of bone marrow B cells. In one embodiment, the B cell repertoire consists essentially of peripheral B cells, splenic B cells, and bone marrow B cells.

[00201] In one aspect, a genetically modified non-human animal is provided, wherein more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or more than 90% of the B cells of the non-human animal that express a heavy chain immunoglobulin variable domain express a heavy chain immunoglobulin variable domain derived from a single V_H gene segment family member. In one embodiment, at least 75% of the B cells of the non-human animal that express an immunoglobulin heavy chain variable domain express an immunoglobulin heavy chain variable domain derived from the single V_H gene segment family member. In a specific embodiment, the percentage is at least 90%. In one embodiment, all of the B cells that express a heavy chain domain that is derived from the single V_H gene family member.

[00202] In one aspect, a genetically modified mouse is provided that makes an antigen-specific B cell population in response to immunization with an antigen of interest, wherein at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or more than 90%, of said antigen-specific B cell population expresses immunoglobulin heavy chains that are all derived from the same V_H gene segment. In one embodiment, at least 75% of the antigen-specific B cell population expresses immunoglobulin heavy chains derived from the same V_H gene segment. In one embodiment, all of the

antigen-specific B cells express a heavy chain that is derived from the same V_H gene segment.

[00203] In one aspect, a non-human animal comprising a restricted V_H gene segment repertoire is provided, wherein the restriction is to a human V_H1-69 gene segment or a V_H1-69 gene segment that is at least about 75.5%, 76.5%, 86.7%, 87.8%, 94.9%, 96.9%, 98%, or 99% identical to a V_H1-69*01 gene segment. In a specific embodiment, the restricted repertoire is selected from one or more of the V_H1-69 variants of FIG. 7.

[00204] In one aspect, a non-human animal comprising a restricted V_H gene segment repertoire is provided, wherein the restriction is to a human V_H1-2 gene segment or a V_H1-2 gene segment that is at least about 94.9%, 95.9%, 96.9%, 98%, or 99% identical to a V_H1-2 gene segment. In a specific embodiment, the restricted repertoire is selected from one or more of the V_H1-2 variants of FIG. 10.

[00205] In one embodiment, the non-human animal is a mouse.

[00206] In one aspect, a non-human animal comprising a restricted human V_H segment repertoire is provided, further comprising a humanized immunoglobulin light chain variable segment locus, wherein the ratio of λ to κ light chains expressed in the mouse is about the same as in a wild-type mouse.

[00207] In one aspect, a non-human animal is provided, comprising a restricted immunoglobulin heavy chain locus characterized by the presence of a single V_H gene segment, one or more D_H gene segments, and one or more J_H gene segments, wherein the single V_H gene segment is a polymorphic V_H gene segment.

[00208] In one embodiment, the polymorphic V_H gene segment is a human V_H gene segment that is associated with a high copy number in human populations. In one embodiment, the human V_H gene segment is selected from V_H1-2, V_H1-69, V_H2-26, V_H2-70, V_H3-23, or a polymorphic variant thereof. In a specific embodiment, the human V_H gene segment is a V_H1-69 gene segment. In another specific embodiment, the human VH gene segment is a V_H1-2 gene segment.

[00209] In one embodiment, the single V_H gene segment is operably linked to a human, mouse, or chimeric human/mouse immunoglobulin constant region gene. In a specific embodiment, the immunoglobulin constant region gene is a mouse constant region gene. In one embodiment, the immunoglobulin constant gene comprises a human sequence selected from a human C_H1, a human hinge, a human C_H2, a human C_H3, and a combination thereof. In one embodiment, the mouse constant gene is at an endogenous immunoglobulin heavy chain locus.

[00210] In one embodiment, the non-human animal further comprises a human immunoglobulin V_L gene segment operably linked to a J gene segment and a light

chain constant gene. In a specific embodiment, the V_L gene segment and/or the J gene segment are selected from a human κ gene segment and a human λ gene segment. In one embodiment, the V_L and/or J gene segments are human κ gene segments.

[00211] In various embodiments, the non-human animal comprises a deletion of all or substantially all endogenous V_H gene segments.

[00212] In various embodiments, the non-human animal comprises an inactivated endogenous heavy chain variable gene locus. In various embodiments, the inactivated endogenous heavy chain variable gene locus is not operably linked to an endogenous heavy chain constant region gene.

[00213] In one aspect, a non-human animal is provided, wherein the non-human animal is characterized by the expression of serum immunoglobulin, wherein greater than 80% of the serum immunoglobulin comprises a human heavy chain variable domain and a cognate human light chain variable domain, wherein the human heavy chain variable domain is derived from a V_H gene segment repertoire consisting essentially of a single human V_H gene segment and/or polymorphic variants thereof.

[00214] In one embodiment, the single human V_H gene segment is a human V_H1-69 gene segment and/or polymorphic variants thereof. In one embodiment, the single human V_H gene segment is a human V_H1-2 gene segment and/or polymorphic variants thereof.

[00215] In one aspect, a non-human animal is provided, comprising, in its germline, a replacement at an endogenous immunoglobulin heavy chain locus of all or substantially all endogenous V_H gene segments with a single human V_H gene segment and/or polymorphic variants thereof.

[00216] In one embodiment, the non-human animal further comprises a replacement at an endogenous immunoglobulin light chain locus of all or substantially all endogenous V_L gene segments with one or more human V_L gene segments. In a specific embodiment, the mouse further comprises one or more human J_L gene segments operably linked to the human V_L gene segments.

[00217] In one aspect, a non-human animal that expresses an antibody that comprises at least one human variable domain/non-human constant domain immunoglobulin polypeptide is provided, wherein the non-human animal expresses a non-human ADAM6 protein or ortholog or homolog thereof from an endogenous immunoglobulin heavy chain locus. In one embodiment, the endogenous immunoglobulin heavy chain locus is incapable of rearranging to encode a functional heavy chain of an antibody.

[00218] In one aspect, a non-human animal that expresses an antibody that comprises at least one human variable domain/non-human constant domain immunoglobulin polypeptide is provided, wherein the non-human animal expresses a non-human ADAM6 protein or ortholog or homolog thereof from a locus other than an immunoglobulin locus.

[00219] In one embodiment, the ADAM6 protein or ortholog or homolog thereof is expressed in a B cell of the non-human animal, wherein the B cell comprises a rearranged immunoglobulin sequence that comprises a human variable sequence and a non-human constant sequence.

[00220] In one embodiment, the non-human constant sequence is a rodent sequence. In one embodiment, the rodent is selected from a mouse, a rat, and a hamster.

[00221] In one aspect, a method is provided for making an infertile male non-human animal, comprising rendering an endogenous ADAM6 allele of a donor ES cell nonfunctional (or knocking out said allele), introducing the donor ES cell into a host embryo, gestating the host embryo in a surrogate mother, and allowing the surrogate mother to give birth to progeny derived in whole or in part from the donor ES cell. In one embodiment, the method further comprises breeding progeny to obtain an infertile male non-human animal.

[00222] In one aspect, a method is provided for making a non-human animal with a genetic modification of interest, wherein the non-human animal is infertile, the method comprising the steps of (a) making a genetic modification of interest in a genome; (b) modifying the genome to knockout an endogenous ADAM6 allele, or render an endogenous ADAM6 allele nonfunctional; and, (c) employing the genome in making a non-human animal. In various embodiments, the genome is from an ES cell or used in a nuclear transfer experiment.

[00223] In one aspect, a non-human animal made using a targeting vector, nucleotide construct, or cell as described herein is provided.

[00224] In one aspect, a progeny of a mating of a non-human animal as described herein with a second non-human animal that is a wild-type non-human animal or genetically modified is provided.

[00225] In one aspect, a method for maintaining a non-human animal strain is provided, wherein the non-human animal strain comprises a replacement of a non-human immunoglobulin heavy chain sequence with one or more heterologous immunoglobulin heavy chain sequences. In one embodiment, the one or more heterologous immunoglobulin heavy chain sequences are human immunoglobulin heavy chain sequences.

[00226] In one embodiment, the non-human animal strain comprises a deletion of one or more non-human V_H, D_H, and/or J_H gene segments. In one embodiment, the non-human animal further comprises a single human V_H gene segment, one or more human D_H gene segments, and/or one or more human J_H gene segments. In one embodiment, the non-human animal comprises a single human V_H segment, at least 27 human D_H gene segments, and at least six J_H gene segments. In a specific embodiment, the non-human animal comprises a single human V_H segment, 27 human D_H gene segments, and six human J_H gene segments, wherein said single human V_H gene segment, 27 human D_H gene segments, and six human J_H gene segments are operably linked to a constant region gene. In one embodiment, the constant region gene is a non-human constant region gene. In one embodiment, the constant region gene comprises a mouse or rat constant region gene sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and/or a C_H4 or a combination thereof. In various embodiments, the single human V_H gene segment is a human V_H1-69 or a human V_H1-2 gene segment.

[00227] In one embodiment, the method comprises generating a male non-human animal heterozygous for the replacement of the non-human immunoglobulin heavy chain sequence, and breeding the heterozygous male non-human animal with a wild-type female non-human animal or a female non-human animal that is homozygous or heterozygous for the human heavy chain sequence. In one embodiment, the method comprises maintaining the non-human animal strain by repeatedly breeding heterozygous males with females that are wild type or homozygous or heterozygous for the human heavy chain sequence.

[00228] In one embodiment, the method comprises obtaining cells from male or female non-human animals homozygous or heterozygous for the human heavy chain sequence, and employing those cells as donor cells or nuclei therefrom as donor nuclei, and using the cells or nuclei to make genetically modified non-human animals using host cells and/or gestating the cells and/or nuclei in surrogate mothers.

[00229] In one embodiment, only male non-human animals that are heterozygous for the replacement at the heavy chain locus are bred to female non-human animals. In a specific embodiment, the female non-human animals are homozygous, heterozygous, or wild type with respect to a replaced heavy chain locus.

[00230] In one embodiment, the non-human animals further comprise a replacement of λ and/or κ light chain variable sequences at an endogenous immunoglobulin light chain locus with heterologous immunoglobulin light chain sequences. In one embodiment, the heterologous immunoglobulin light chain sequences are human immunoglobulin λ and/or κ light chain variable sequences.

[00231] In one embodiment, the non-human animal further comprises a transgene at a locus other than an endogenous immunoglobulin locus, wherein the transgene comprises a sequence encoding a rearranged or unrearranged heterologous λ or κ light chain sequence (*e.g.*, unrearranged V_L and unrearranged J_L, or rearranged V_LJ_L) operably linked (for unrearranged) or fused (for rearranged) to an immunoglobulin light chain constant region sequence. In one embodiment, the heterologous λ or κ light chain sequence is human. In one embodiment, the constant region sequence is selected from rodent, human, and non-human primate. In one embodiment, the constant region sequence is selected from mouse, rat, and hamster. In one embodiment, the transgene comprises a non-immunoglobulin promoter that drives expression of the light chain sequences. In a specific embodiment, the promoter is a transcriptionally active promoter. In a specific embodiment, the promoter is a ROSA26 promoter.

[00232] In one aspect, a method for making a genetically modified non-human animal is provided, comprising inserting a non-human nucleotide sequence that comprises a non-human immunoglobulin gene segment in the genome of the animal for a first modification, wherein the insertion maintains an endogenous ADAM6 gene, then rendering the endogenous immunoglobulin heavy chain locus of the non-human animal non-functional for a second modification. In one embodiment, the first modification is performed upstream of an endogenous immunoglobulin heavy chain constant region gene and the second modification is performed to invert, translocate, or place out of operable linkage the endogenous immunoglobulin heavy chain locus such that the endogenous immunoglobulin heavy chain locus is incapable of rearranging to encode a functional heavy chain variable region.

[00233] In one aspect, a method for making a genetically modified non-human animal is provided, comprising replacing a non-human nucleotide sequence that comprises a non-human immunoglobulin gene segment and a non-human ADAM6 (or ortholog or homolog or fragment thereof functional in a male non-human animal) nucleotide sequence with a sequence comprising a human immunoglobulin gene segment to form a first chimeric locus, then inserting a sequence comprising a non-human ADAM6-encoding sequence (or a sequence encoding an ortholog or homolog or functional fragment thereof) into the sequence comprising the human immunoglobulin gene segment to form a second chimeric locus.

[00234] In one embodiment, the second chimeric locus comprises a human immunoglobulin heavy chain variable (V_H) gene segment. In one embodiment, the second chimeric locus comprises a human immunoglobulin light chain variable (V_L)

gene segment. In a specific embodiment, the second chimeric locus comprises a human V_H gene segment or a human V_L gene segment operably linked to a human D_H gene segment and a human J_H gene segment. In a further specific embodiment, the second chimeric locus is operably linked to a third chimeric locus that comprises a human C_H1 sequence, or a human C_H1 and human hinge sequence, fused with a mouse C_H2 + C_H3 sequence.

[00235] In one aspect, use of a mouse that comprises an ectopic nucleotide sequence comprising a mouse ADAM6 locus or sequence to make a fertile male mouse is provided, wherein the use comprises mating the mouse comprising the ectopic nucleotide sequence that comprises the mouse ADAM6 locus or sequence to a mouse that lacks a functional endogenous mouse ADAM6 locus or sequence, and obtaining a progeny that is a female capable of producing progeny having the ectopic ADAM6 locus or sequence or that is a male that comprises the ectopic ADAM6 locus or sequence, and the male exhibits a fertility that is approximately the same as a fertility exhibited by a wild-type male mouse.

[00236] In one aspect, use of a mouse as described herein to make an immunoglobulin variable region nucleotide sequence is provided.

[00237] In one aspect, use of a mouse as described herein to make a fully human Fab or a fully human $F(ab)_2$ is provided.

[00238] In one aspect, use of a mouse as described herein to make an immortalized cell line is provided.

[00239] In one aspect, use of a mouse as described herein to make a hybridoma or quadroma is provided.

[00240] In one aspect, use of a mouse as described herein to make a phage library containing human heavy chain variable regions and human light chain variable regions is provided.

[00241] In one embodiment, the human heavy chain variable regions are derived from a human V_H1-69 gene segment that comprises a sequence selected from SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57 and SEQ ID NO: 59.

[00242] In one embodiment, the human heavy chain variable regions are derived from a human V_H1-69 gene segment that comprises a sequence selected from SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60 and SEQ ID NO: 62.

[00243] In one embodiment, the human heavy chain variable regions are all

derived from a human V_H1-2 gene segment that comprises a sequence selected from SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69 and SEQ ID NO: 71.

[00244] In one embodiment, the human heavy chain variable regions are derived from a human V_H1-2 gene segment that comprises a sequence selected from SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70 and SEQ ID NO: 72.

[00245] In one aspect, use of a mouse as described herein to generate a variable region sequence for making a human antibody is provided, comprising (a) immunizing a mouse as described herein with an antigen of interest, (b) isolating a lymphocyte from the immunized mouse of (a), (c) exposing the lymphocyte to one or more labeled antibodies, (d) identifying a lymphocyte that is capable of binding to the antigen of interest, and (e) amplifying one or more variable region nucleic acid sequence from the lymphocyte thereby generating a variable region sequence.

[00246] In one embodiment, the lymphocyte is derived from the spleen of the mouse. In one embodiment, the lymphocyte is derived from a lymph node of the mouse. In one embodiment, the lymphocyte is derived from the bone marrow of the mouse.

[00247] In one embodiment, the labeled antibody is a fluorophore-conjugated antibody. In one embodiment, the one or more fluorophore-conjugated antibodies are selected from an IgM, an IgG, and/or a combination thereof.

[00248] In one embodiment, the lymphocyte is a B cell.

[00249] In one embodiment, the one or more variable region nucleic acid sequence comprises a heavy chain variable region sequence. In one embodiment, the one or more variable region nucleic acid sequence comprises a light chain variable region sequence. In a specific embodiment, the light chain variable region sequence is an immunoglobulin κ light chain variable region sequence. In one embodiment, the one or more variable region nucleic acid sequence comprises a heavy chain and a κ light chain variable region sequence.

[00250] In one embodiment, use of a mouse as described herein to generate a heavy and a κ light chain variable region sequence for making a human antibody is provided, comprising (a) immunizing a mouse as described herein with an antigen of interest, (b) isolating the spleen from the immunized mouse of (a), (c) exposing B lymphocytes from the spleen to one or more labeled antibodies, (d) identifying a B lymphocyte of (c) that is capable of binding to the antigen of interest, and (e) amplifying a heavy chain variable region nucleic acid sequence and a κ light chain variable region nucleic acid sequence from the B lymphocyte thereby generating the

heavy chain and κ light chain variable region sequences.

[00251] In one embodiment, use of a mouse as described herein to generate a heavy and a κ light chain variable region sequence for making a human antibody is provided, comprising (a) immunizing a mouse as described herein with an antigen of interest, (b) isolating one or more lymph nodes from the immunized mouse of (a), (c) exposing B lymphocytes from the one or more lymph nodes to one or more labeled antibodies, (d) identifying a B lymphocyte of (c) that is capable of binding to the antigen of interest, and (e) amplifying a heavy chain variable region nucleic acid sequence and a κ light chain variable region nucleic acid sequence from the B lymphocyte thereby generating the heavy chain and κ light chain variable region sequences.

[00252] In one embodiment, use of a mouse as described herein to generate a heavy and a κ light chain variable region sequence for making a human antibody is provided, comprising (a) immunizing a mouse as described herein with an antigen of interest, (b) isolating bone marrow from the immunized mouse of (a), (c) exposing B lymphocytes from the bone marrow to one or more labeled antibodies, (d) identifying a B lymphocyte of (c) that is capable of binding to the antigen of interest, and (e) amplifying a heavy chain variable region nucleic acid sequence and a κ light chain variable region nucleic acid sequence from the B lymphocyte thereby generating the heavy chain and κ light chain variable region sequences. In various embodiments, the one or more labeled antibodies are selected from an IgM, an IgG, and/or a combination thereof.

[00253] In various embodiments, the antigen of interest is a pathogen that afflicts human subjects including, *e.g.,* a viral antigen. Exemplary viral pathogens include, *e.g.,* mainly those of the families of Adenoviridae, bacteria Picornaviridae, Herpesviridae, Hepadnaviridae, Flaviviridae, Retroviridae, Orthomyxoviridae, Paramyxoviridae, Papovaviridae, Polyomavirus, Rhabdoviridae, and Togaviridae. Such exemplary viruses typically range between 20-300 nanometers in length. In various embodiments, the antigen of interest is a viral antigen selected from a hepatitis virus (*e.g.*, HCV, HBV, *etc.*), a human immunodeficiency virus (HIV), or an influenza virus.

[00254] In various embodiments, use of a mouse as described herein to generate a heavy and κ light chain variable region sequence for making a human antibody is provided, further comprising fusing the amplified heavy and light chain variable region sequences to human heavy and light chain constant region sequences, expressing the fused heavy and light chain sequences in a cell, and recovering the

expressed heavy and light chain sequences thereby generating a human antibody.

[00255] In various embodiments, the human heavy chain constant regions are selected from IgM, IgD, IgA, IgE and IgG. In various specific embodiments, the IgG is selected from an IgG1, an IgG2, an IgG3 and an IgG4. In various embodiments, the human heavy chain constant region comprises a C_H1, a hinge, a C_H2, a C_H3, a C_H4, or a combination thereof. In various embodiments, the light chain constant region is an immunoglobulin κ constant region. In various embodiments, the cell is selected from a HeLa cell, a DU145 cell, a Lncap cell, a MCF-7 cell, a MDA-MB-438 cell, a PC3 cell, a T47D cell, a THP-1 cell, a U87 cell, a SHSY5Y (human neuroblastoma) cell, a Saos-2 cell, a Vero cell, a CHO cell, a GH3 cell, a PC12 cell, a human retinal cell (*e.g.*, a PER.C6™ cell), and a MC3T3 cell. In a specific embodiment, the cell is a CHO cell.

[00256] In one aspect, a method for generating a reverse-chimeric rodent-human antibody specific against an antigen of interest is provided, comprising the steps of immunizing a mouse as described herein with the antigen, isolating at least one cell from the mouse producing a reverse-chimeric mouse-human antibody specific against the antigen, culturing at least one cell producing the reverse-chimeric mouse-human antibody specific against the antigen, and obtaining said antibody.

[00257] In one embodiment, the reverse-chimeric mouse-human antibody comprises a human heavy chain variable domain fused with a mouse or rat heavy chain constant gene, and a human light chain variable domain fused with a mouse or rat or human light chain constant gene. In a specific embodiment, the human heavy chain variable domain contains a rearranged human V_H1-69 or human V_H1-2 gene segment.

[00258] In one embodiment, culturing at least one cell producing the reverse-chimeric rodent-human antibody specific against the antigen is performed on at least one hybridoma cell generated from the at least one cell isolated from the mouse.

[00259] In one aspect, a method for generating a fully human antibody specific against an antigen of interest is provided, comprising the steps of immunizing a mouse as described herein with the antigen, isolating at least one cell from the mouse producing a reverse-chimeric rodent-human antibody specific against the antigen, generating at least one cell producing a fully human antibody derived from the reverse-chimeric rodent-human antibody specific against the antigen, and culturing at least one cell producing the fully human antibody, and obtaining said fully human antibody.

[00260] In various embodiments, the at least one cell isolated from the mouse producing a reverse-chimeric rodent-human antibody specific against the antigen is a

splenocyte or a B cell.

[00261] In various embodiments, the antibody is a monoclonal antibody.

[00262] In various embodiments, the antibody comprises a heavy chain variable domain that contains a rearranged human V_H1-69 or human V_H1-2 gene segment.

[00263] In various embodiments, immunization with the antigen of interest is carried out with protein, DNA, a combination of DNA and protein, or cells expressing the antigen.

[00264] In one aspect, use of a mouse as described herein to make a nucleic acid sequence encoding an immunoglobulin variable region or fragment thereof is provided. In one embodiment, the nucleic acid sequence is used to make a human antibody or antigen-binding fragment thereof. In one embodiment, the mouse is used to make an antigen-binding protein selected from an antibody, a multi-specific antibody (*e.g.*, a bi-specific antibody), an scFv, a bi-specific scFv, a diabody, a triabody, a tetrabody, a V-NAR, a V_{HH}, a V_L, a F(ab), a $F(ab)_2$, a DVD (*i.e.*, dual variable domain antigen-binding protein), a an SVD (*i.e.*, single variable domain antigen-binding protein), or a bispecific T-cell engager (BiTE).

[00265] In one aspect, a method for making a human antigen-binding protein is provided, comprising exposing a genetically engineered non-human animal as described herein to an antigen of interest, allowing the non-human animal to mount an immune response to the antigen, obtaining from the non-human animal a heavy chain variable domain nucleic acid sequence encoding a human heavy chain variable domain that specifically binds the antigen of interest, fusing the heavy chain variable domain nucleic acid sequence to a human constant region sequence, and expressing in a mammalian cell an antibody comprising the human heavy chain variable domain sequence and the human constant region sequence. In one embodiment, the mammalian cell is a CHO cell. In one embodiment the non-human animal comprises a human V_H gene segment repertoire that consists essentially of a single human V_H gene segment, optionally present in two or more polymorphic variants thereof, operably linked to one or more human D and/or J segments. In one embodiment, the human V_H gene segment repertoire is at an endogenous non-human V_H gene segment locus. In one embodiment, the human V_H gene segment repertoire is at a locus that is not an endogenous V_H gene segment locus. In one embodiment, the human V_H gene segment rearranges with a human D segment and a human J segment to form a rearranged human VDJ gene operably linked to a constant region sequence, wherein the constant region sequence is selected from a human sequence and a rodent sequence (*e.g.*, a mouse or rat or hamster sequence). In one embodiment, the constant region sequence comprises a

sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof; in a specific embodiment, the constant region sequence comprises a C_H1, a hinge, a C_H2, and a C_H3. In one embodiment, the human variable domain and the constant sequence are expressed in the mammalian cell with a cognate human light chain variable domain obtained from the same mouse (*e.g.*, sequence obtained from the same B cell as the human variable domain sequence); in one embodiment the sequence encoding the human light chain variable domain obtained from the mouse is then fused with a sequence encoding a human light chain constant sequence, and the light chain sequence and the heavy chain sequence are expressed in the mammalian cell.

[00266] In one aspect, a method for making an antibody heavy chain variable domain that binds an antigen of interest is provided, comprising expressing in a single cell (a) a first V_H sequence of an immunized non-human animal as described herein, wherein the first V_H sequence is fused with a C_H gene sequence; and (b) a V_L gene sequence of an immunized non-human animal as described herein, wherein the V_L gene sequence is fused with a human C_L gene sequence; maintaining the cell under conditions sufficient to express an antibody; and, isolating the antibody heavy chain variable domain. In one embodiment, the V_L gene sequence is cognate with the first V_H sequence.

[00267] In one embodiment, the cell comprises a second V_H gene sequence of an immunized non-human animal as described herein, wherein the second V_H gene sequence is fused with a C_H gene sequence, wherein the first V_H gene sequence encodes a V_H domain that specifically binds a first epitope, and the second V_H gene sequence encodes a V_H domain that specifically binds a second epitope, wherein the first epitope and the second epitope are not identical.

[00268] In one embodiment, the constant region sequences are all human constant region sequences.

[00269] In one aspect, a method for making a human bispecific antibody is provided, comprising making the bispecific antibody using human variable region gene sequences of B cells of a non-human animal as described herein.

[00270] In one embodiment, the method comprises (a) identifying a clonally selected lymphocyte of the non-human animal, wherein the non-human animal has been exposed to an antigen of interest and allowed to develop an immune response to the antigen of interest, and wherein the lymphocyte expresses an antibody that specifically binds the antigen of interest, (b) obtaining from the lymphocyte or the antibody a nucleotide sequence that encodes a human heavy chain variable region that specifically binds the antigen of interest, and (c) employing the nucleotide

sequence that encodes the human heavy chain variable region that specifically binds the antigen of interest in making the bispecific antibody. In a specific embodiment, the human heavy chain variable region comprises a rearranged V_H1-2 or V_H1-69 gene segment.

[00271] In one embodiment, steps (a) through (c) are performed a first time for a first antigen of interest to generate a first human heavy chain variable region sequence, and steps (a) through (c) are performed a second time for a second antigen of interest to generate a second human heavy chain variable region sequence, and wherein the first human heavy chain variable region sequence is expressed fused with a first human heavy chain constant region to form a first human heavy chain, the second human heavy chain variable region sequence is expressed fused with a second human heavy chain constant region to form a second human heavy chain, wherein the first and the second human heavy chains are expressed in the presence of a single human light chain expressed from a rearranged human Vκ1-39 or a human Vκ3-20 gene segment. In a specific embodiment, the single human light chain comprises a germline sequence.

[00272] In one embodiment, the method comprises (a) cloning heavy chain variable regions from B cells of a non-human animal as described herein which has been exposed to a first antigen of interest, and the same non-human animal, or a different non-human animal which is genetically the same and has been exposed to a second antigen of interest; and (b) expressing in a cell the heavy chain variable regions of (a) with the same heavy chain constant region and the same light chain to make a bispecific antibody.

[00273] In one aspect, a use of a non-human animal as described herein is provided, to obtain a nucleic acid sequence that encodes a human heavy chain variable domain. In one embodiment, the heavy chain variable domain comprises a rearranged human V_H gene segment selected from V_H1-2 and V_H1-69.

[00274] In one aspect, a use of a non-human animal as described herein is provided, to obtain a cell that encodes a human heavy chain variable domain. In one embodiment, the heavy chain variable domain comprises a rearranged human V_H gene segment selected from V_H1-2 and V_H1-69.

[00275] In one aspect, use of a non-human animal as described herein to make a human antibody variable domain is provided. In one aspect, use of a non-human animal as described herein to make a human antibody is provided. In one embodiment, the human antibody is a human bispecific antibody. In various embodiments, the variable domain and/or the antibody comprises a rearranged

human V_H gene segment selected from V_H1-2 and V_H1-69.

[00276] In one aspect, use of a non-human animal as described herein is provided to select a human immunoglobulin heavy chain variable domain. In one embodiment, the heavy chain variable domain comprises a rearranged human V_H gene segment selected from V_H1-2 and V_H1-69.

[00277] In one aspect, use of a mouse as described herein to introduce an ectopic ADAM6 sequence into a mouse that lacks a functional endogenous mouse ADAM6 sequence is provided, wherein the use comprises mating a mouse as described herein with the mouse that lacks the functional endogenous mouse ADAM6 sequence.

[00278] In one aspect, use of genetic material from a mouse as described herein to make a mouse having an ectopic ADAM6 sequence is provided. In one embodiment, the *use* comprises nuclear transfer using a nucleus of a cell of a mouse as described herein. In one embodiment, the use comprises cloning a cell of a mouse as described herein to produce an animal derived from the cell. In one embodiment, the use comprises employing a sperm or an egg of a mouse as described herein in a process for making a mouse comprising the ectopic ADAM6 sequence.

[00279] In one aspect, a method for making a fertile male mouse comprising a modified immunoglobulin heavy chain locus is provided, comprising fertilizing a first mouse germ cell that comprises a modification of an endogenous immunoglobulin heavy chain locus with a second mouse germ cell that comprises an ADAM6 gene or ortholog or homolog or fragment thereof that is functional in a male mouse; forming a fertilized cell; allowing the fertilized cell to develop into an embryo; and, gestating the embryo in a surrogate to obtain a mouse.

[00280] In one embodiment, the fertilization is achieved by mating a male mouse and a female mouse. In one embodiment, the female mouse comprises the ADAM6 gene or ortholog or homolog or fragment thereof. In one embodiment, the male mouse comprises the ADAM6 gene or ortholog or homolog or fragment thereof.

[00281] In one aspect, use of a nucleic acid sequence encoding a mouse ADAM6 protein or an ortholog or homolog thereof or a functional fragment of the corresponding ADAM6 protein for restoring or enhancing the fertility of a mouse having a genome comprising a modification of an immunoglobulin heavy chain locus is provided, wherein the modification reduces or eliminates endogenous ADAM6 function.

[00282] In one embodiment, the nucleic acid sequence is integrated into the genome of the mouse at an ectopic position. In one embodiment, the nucleic acid

sequence is integrated into the genome of the mouse at an endogenous immunoglobulin locus. In a specific embodiment, the endogenous immunoglobulin locus is a heavy chain locus. In one embodiment, the nucleic acid sequence is integrated into the genome of the mouse at a position other than an endogenous immunoglobulin locus.

[00283] In one aspect, use of the mouse as described herein for the manufacture of a medicament (*e.g.*, an antigen-binding protein), or for the manufacture of a sequence encoding a variable sequence of a medicament (*e.g.*, an antigen-binding protein), for the treatment of a human disease or disorder is provided. In one embodiment, the variable sequence of a medicament comprises a polymorphic human V_H gene segment. In one embodiment, the variable sequence of a medicament comprises a human V_H1-69 gene segment. In one embodiment, the variable sequence of a medicament comprises a human V_H1-2 gene segment.

[00284] In one aspect, a nucleic acid construct encoding an immunoglobulin variable domain made in a mouse as described herein is provided. In one embodiment, the variable domain is a heavy chain variable domain. In a specific embodiment, the heavy chain variable domain comprises a human V_H gene segment selected from V_H1-2, V_H1-69, V_H2-26, V_H2-70, or V_H3-23. In another specific embodiment, the heavy chain variable domain comprises a human V_H1-2 gene segment. In another specific embodiment, the heavy chain variable domain comprises a human V_H1-69 gene segment.

[00285] In one embodiment, the variable domain is a light chain variable domain. In a specific embodiment, the variable domain is a κ light chain variable domain that is cognate with a human heavy chain variable domain that comprises a rearranged human V_H1-69 gene segment. In a specific embodiment, the variable domain is a κ light chain variable domain that is cognate with a human heavy chain variable domain that comprises a rearranged human V_H1-2 gene segment.

[00286] In one aspect, use of a mouse as described herein to make a nucleic acid construct encoding a human immunoglobulin variable domain is provided. In one embodiment, the variable domain is a light chain variable domain. In one embodiment, the variable domain is a κ light chain variable domain that comprises a rearranged human Vκ gene segment selected from Vκ4-1, Vκ5-2, Vκ7-3, Vκ2-4, Vκ1-5, Vκ1-6, Vκ3-7, Vκ1-8, Vκ1-9, Vκ2-10, Vκ3-11, Vκ1-12, Vκ1-13, Vκ2-14, Vκ3-15, Vκ1-16, Vκ1-17, Vκ2-18, Vκ2-19, Vκ3-20, Vκ6-21, Vκ1-22, Vκ1-23, Vκ2-24, Vκ3-25, Vκ2-26, Vκ1-27, Vκ2-28, Vκ2-29, Vκ2-30, Vκ3-31, Vκ1-32, Vκ1-33, Vκ3-34, Vκ1-35, Vκ2-36, Vκ1-37, Vκ2-38, Vκ1-39, and Vκ2-40.

[00287] In one embodiment, the variable domain is a heavy chain variable domain. In a specific embodiment, the heavy chain variable domain comprises a rearranged human V_H gene segment selected from V_H1-2, V_H1-69, V_H2-26, V_H2-70, or V_H3-23. In a specific embodiment, the heavy chain variable domain comprises a rearranged human V_H1-69 gene segment. In a specific embodiment, the heavy chain variable domain comprises a rearranged human V_H1-2 gene segment.

[00288] In one aspect, use of a mouse as described herein to make a human immunoglobulin variable domain is provided. In one embodiment, the variable domain is a light chain variable domain. In one embodiment, the variable domain is a κ light chain variable domain that comprises a rearranged human Vκ gene segment selected from Vκ4-1, Vκ5-2, Vκ7-3, Vκ2-4, Vκ1-5, Vκ1-6, Vκ3-7, Vκ1-8, Vκ1-9, Vκ2-10, Vκ3-11, Vκ1-12, Vκ1-13, Vκ2-14, Vκ3-15, Vκ1-16, Vκ1-17, Vκ2-18, Vκ2-19, Vκ3-20, Vκ6-21, Vκ1-22, Vκ1-23, Vκ2-24, Vκ3-25, Vκ2-26, Vκ1-27, Vκ2-28, Vκ2-29, Vκ2-30, Vκ3-31, Vκ1-32, Vκ1-33, Vκ3-34, Vκ1-35, Vκ2-36, Vκ1-37, Vκ2-38, Vκ1-39, and Vκ2-40.

[00289] In one embodiment, the variable domain is a heavy chain variable domain. In a specific embodiment, the heavy chain variable domain comprises a rearranged human V_H gene segment selected from V_H1-2, V_H1-69, V_H2-26, V_H2-70, or V_H3-23. In a specific embodiment, the heavy chain variable domain comprises a rearranged human V_H1-69 gene segment. In a specific embodiment, the heavy chain variable domain comprises a rearranged human V_H1-2 gene segment.

[00290] The various aspects and embodiments are capable of use together, unless expressly noted otherwise or the context clearly prohibits use together.

BRIEF DESCRIPTION OF FIGURES

[00291] **FIG. 1** shows a general illustration, not to scale, of a series of targeting and molecular engineering steps employed to make a targeting vector for construction of a modified heavy chain locus containing a single human V_H1-69 gene segment, twenty-seven human D_H and six human J_H gene segments at an endogenous immunoglobulin heavy chain locus.

[00292] **FIG. 2** shows a general illustration, not to scale, of a series of targeting and molecular engineering steps employed to make a targeting vector for construction of a modified heavy chain locus containing a single human V_H1-2 gene segment, twenty-seven human D_H and six human J_H gene segments at an endogenous immunoglobulin heavy chain locus.

[00293] **FIG. 3** shows a general illustration, not to scale, of a series of targeting and molecular engineering steps employed to make a targeting vector for construction of a modified heavy chain locus containing a single human V_H1-69 gene segment, twenty-seven human D_H, six human J_H gene segments and an ectopic genomic fragment encoding mouse ADAM6 at an endogenous immunoglobulin heavy chain locus.

[00294] **FIG. 4** shows a general illustration, not to scale, of a series of targeting and molecular engineering steps employed to make a targeting vector for construction of a modified heavy chain locus containing a single human V_H1-2 gene segment, twenty-seven human D_H, six human J_H gene segments and an ectopic genomic fragment encoding mouse ADAM6 at an endogenous immunoglobulin heavy chain locus.

[00295] **FIG. 5** shows the nucleotide alignment of the second exon for each of thirteen reported alleles for the human V_H1-69 gene. Lower case bases indicate germline nucleotide differences among the alleles. Complementary determining regions (CDRs) are indicated with boxes around the sequence. Dashes indicate artificial gaps for proper sequence alignment. V_H1-69*01 (SEQ ID NO: 37); V_H1-69*02 (SEQ ID NO: 39); V_H1-69*03 (SEQ ID NO: 41); V_H1-69*04 (SEQ ID NO: 43); V_H1-69*05 (SEQ ID NO: 45); V_H1-69*06 (SEQ ID NO: 47); V_H1-69*07 (SEQ ID NO: 49); V_H1-69*08 (SEQ ID NO: 51); V_H1-69*09 (SEQ ID NO: 53); V_H1-69*10 (SEQ ID NO: 55); V_H1-69*11 (SEQ ID NO: 57); V_H1-69*12 (SEQ ID NO: 59); V_H1-69*13 (SEQ ID NO: 61).

[00296] **FIG. 6** shows the protein alignment of the mature heavy chain variable gene sequence for each of thirteen reported alleles for the human V_H1-69 gene. Lower case amino acids indicate germline differences among the alleles. Complementary determining regions (CDRs) are indicated with boxes around the sequence. Dashes indicate artificial gaps for proper sequence alignment. V_H1-69*01 (SEQ ID NO: 38); V_H1-69*02 (SEQ ID NO: 40); V_H1-69*03 (SEQ ID NO: 42); V_H1-69*04 (SEQ ID NO: 44); V_H1-69*05 (SEQ ID NO: 46); V_H1-69*06 (SEQ ID NO: 48); V_H1-69*07 (SEQ ID NO: 50); V_H1-69*08 (SEQ ID NO: 52); V_H1-69*09 (SEQ ID NO: 54); V_H1-69*10 (SEQ ID NO: 56); V_H1-69*11 (SEQ ID NO: 58); V_H1-69*12 (SEQ ID NO: 60); V_H1-69*13 (SEQ ID NO: 62).

[00297] **FIG. 7** shows a percent identity/percent similarity matrix for the aligned protein sequences of the mature variable gene for each of thirteen reported alleles for the human V_H1-69 gene. Percent identity among the V_H1-69 alleles is indicated above the shaded boxes and percent similarity is indicated below the shaded boxes.

Scores for percent identity and percent similarity were scored by a ClustalW (v1.83) alignment tool using MacVector software (MacVector, Inc., North Carolina).

[00298] **FIG. 8** shows the nucleotide alignment of the second exon for each of five reported alleles for the human V_H1-2 gene. Lower case bases indicate germline nucleotide differences among the alleles. Complementary determining regions (CDRs) are indicated with boxes around the sequence. Dashes indicate artificial gaps for proper sequence alignment. V_H1-2*01 (SEQ ID NO: 63); V_H1-2*02 (SEQ ID NO: 65); V_H1-2*03 (SEQ ID NO: 67); V_H1-2*04 (SEQ ID NO: 69); V_H1-2*05 (SEQ ID NO: 71).

[00299] **FIG. 9** shows the protein alignment of the mature heavy chain variable gene sequence for each of the five reported alleles for the human V_H1-2 gene. Lower case amino acids indicate germline differences among the alleles. Complementary determining regions (CDRs) are indicated with boxes around the sequence. Dashes indicate artificial gaps for proper sequence alignment. V_H1-2*01 (SEQ ID NO: 64); V_H1-2*02 (SEQ ID NO: 66); V_H1-2*03 (SEQ ID NO: 68); V_H1-2*04 (SEQ ID NO: 70); V_H1-2*05 (SEQ ID NO: 72).

[00300] **FIG. 10** shows a percent identity/percent similarity matrix for the aligned protein sequences of the mature variable gene for each of five reported alleles for the human V_H1-2 gene. Percent identity among the V_H1-2 alleles is indicated above the shaded boxes and percent similarity is indicated below the shaded boxes. Scores for percent identity and percent similarity were scored by a ClustalW (v1.83) alignment tool using MacVector software (MacVector, Inc., North Carolina).

DETAILED DESCRIPTION

[00301] This invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention is defined by the claims.

[00302] Unless defined otherwise, all terms and phrases used herein include the meanings that the terms and phrases have attained in the art, unless the contrary is clearly indicated or clearly apparent from the context in which the term or phrase is used. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, particular methods and materials are now described.

[00303] The phrase "substantial" or "substantially" when used to refer to an amount of gene segments (*e.g.*, "substantially all" V gene segments) includes both

functional and non functional gene segments and include, in various embodiments, *e.g.*, 80% or more, 85% or more, 90% or more, 95% or more 96% or more, 97% or more, 98% or more, or 99% or more of all gene segments; in various embodiments, "substantially all" gene segments includes, *e.g.*, at least 95%, 96%, 97%, 98%, or 99% of functional (*i.e.*, non-pseudogene) gene segments.

[00304] The term "replacement" includes wherein a DNA sequence is placed into a genome of a cell in such a way as to replace a sequence within the genome with a heterologous sequence (*e.g.*, a human sequence in a mouse), at the locus of the genomic sequence,. The DNA sequence so placed may include one or more regulatory sequences that are part of source DNA used to obtain the sequence so placed (*e.g.*, promoters, enhancers, 5'- or 3'-untranslated regions, appropriate recombination signal sequences, *etc.*). For example, in various embodiments, the replacement is a substitution of an endogenous sequence for a heterologous sequence that results in the production of a gene product from the DNA sequence so placed (comprising the heterologous sequence), but not expression of the endogenous sequence; the replacement is of an endogenous genomic sequence with a DNA sequence that encodes a protein that has a similar function as a protein encoded by the endogenous genomic sequence (*e.g.*, the endogenous genomic sequence encodes an immunoglobulin gene or domain, and the DNA fragment encodes one or more human immunoglobulin genes or domains). In various embodiments, an endogenous gene or fragment thereof is replaced with a corresponding human gene or fragment thereof. A corresponding human gene or fragment thereof is a human gene or fragment that is an ortholog of, a homolog of, or is substantially identical or the same in structure and/or function, as the endogenous gene or fragment thereof that is replaced.

[00305] The mouse as a genetic model has been greatly enhanced by transgenic and knockout technologies, which have allowed for the study of the effects of the directed over-expression or deletion of specific genes. Despite all of its advantages, the mouse still presents genetic obstacles that render it an imperfect model for human diseases and an imperfect platform to test human therapeutics or make them. First, although about 99% of human genes have a mouse homolog (Waterston *et al.* 2002, Initial sequencing and comparative analysis of the mouse genome, *Nature* 420:520-562), potential therapeutics often fail to cross-react, or cross-react inadequately, with mouse orthologs of the intended human targets. To obviate this problem, selected target genes can be "humanized," that is, the mouse gene can be eliminated and replaced by the corresponding human orthologous gene sequence (*e.g.*, US 6,586,251, US 6,596,541 and US 7,105,348). Initially, efforts to humanize

mouse genes by a "knockout-plus-transgenic humanization" strategy entailed crossing a mouse carrying a deletion (i.e., knockout) of the endogenous gene with a mouse carrying a randomly integrated human transgene (see, *e.g.*, Bril *et al.*, 2006, Tolerance to factor VIII in a transgenic mouse expressing human factor VIII cDNA carrying an Arg(593) to Cys substitution, *Thromb Haemost* 95:341-347; Homanics *et al.*, 2006, Production and characterization of murine models of classic and intermediate maple syrup urine disease, *BMC Med Genet* 7:33; Jamsai *et al.*, 2006, A humanized BAC transgenic/knockout mouse model for HbE/beta-thalassemia, *Genomics* 88(3):309-15; Pan *et al.*, 2006, Different role for mouse and human CD3delta/epsilon heterodimer in preT cell receptor (preTCR) function:human CD3delta/epsilon heterodimer restores the defective preTCR function in CD3gamma- and CD3gammadelta-deficient mice, *Mol Immunol* 43:1741-1750). But those efforts were hampered by size limitations; conventional knockout technologies were not sufficient to directly replace large mouse genes with their large human genomic counterparts. A straightforward approach of direct homologous replacement, in which an endogenous mouse gene is directly replaced by the human counterpart gene at the same precise genetic location of the mouse gene (*i.e.*, at the endogenous mouse locus), is rarely attempted because of technical difficulties. Until now, efforts at direct replacement involved elaborate and burdensome procedures, thus limiting the length of genetic material that could be handled and the precision with which it could be manipulated.

[00306] Exogenously introduced human immunoglobulin transgenes rearrange in precursor B cells in mice (Alt *et al.*, 1985, Immunoglobulin genes in transgenic mice, *Trends Genet* 1:231-236). This finding was exploited by engineering mice using the knockout-plus-transgenic approach to express human antibodies (Green *et al.*, 1994, Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs, *Nat Genet* 7:13-21; Lonberg *et al.*, 1994, Antigen-specific human antibodies from mice comprising four distinct genetic modifications, *Nature* 368:856-859; Jakobovits *et al.*, 2007, From XenoMouse technology to panitumumab, the first fully human antibody product from transgenic mice, *Nat Biotechnol* 25:1134-1143). The mouse immunoglobulin heavy chain and κ light chain loci were inactivated in these mice by targeted deletion of small but critical portions of each endogenous locus, followed by introducing human immunoglobulin gene loci as randomly integrated large transgenes, as described above, or minichromosomes (Tomizuka *et al.*, 2000, Double trans-chromosomic mice: maintenance of two individual human chromosome fragments containing Ig heavy

and kappa loci and expression of fully human antibodies, *PNAS USA* 97:722-727). Such mice represented an important advance in genetic engineering; fully human monoclonal antibodies isolated from them yielded promising therapeutic potential for treating a variety of human diseases (Gibson *et al.*, 2006, Randomized phase III trial results of panitumumab, a fully human anti-epidermal growth factor receptor monoclonal antibody, in metastatic colorectal cancer, *Clin Colorectal Cancer* 6:29-31; Jakobovits *et al.*, 2007; Kim *et al.*, 2007, Clinical efficacy of zanolimumab (HuMax-CD4): two Phase II studies in refractory cutaneous T-cell lymphoma, *Blood* 109(11):4655-62; Lonberg, 2005, Human antibodies from transgenic animals, *Nat Biotechnol* 23:1117-1125; Maker *et al.*, 2005, Tumor regression and autoimmunity in patients treated with cytotoxic T lymphocyte-associated antigen 4 blockade and interleukin 2: a phase I/II study, *Ann Surg Oncol* 12:1005-1016; McClung *et al.*, 2006, Denosumab in postmenopausal women with low bone mineral density, *New Engl J Med* 354:821-831). But, as discussed above, these mice exhibit compromised B cell development and immune deficiencies when compared to wild type mice. Such problems potentially limit the ability of the mice to support a vigorous humoral response and, consequently, generate fully human antibodies against some antigens. The deficiencies may be due to inefficient functionality due to the random introduction of the human immunoglobulin transgenes and resulting incorrect expression due to a lack of upstream and downstream control elements (Garrett *et al.*, 2005, Chromatin architecture near a potential 3' end of the IgH locus involves modular regulation of histone modifications during B-Cell development and in vivo occupancy at CTCF sites, *Mol Cell Biol* 25:1511-1525; Manis *et al.*, 2003, Elucidation of a downstream boundary of the 3' IgH regulatory region, *Mol Immunol* 39:753-760; Pawlitzky *et al.*, 2006, Identification of a candidate regulatory element within the 5' flanking region of the mouse IgH locus defined by pro-B cell-specific hypersensitivity associated with binding of PU.1, Pax5, and E2A, *J Immunol* 176:6839-6851), inefficient interspecies interactions between human constant domains and mouse components of the B-cell receptor signaling complex on the cell surface, which may impair signaling processes required for normal maturation, proliferation, and survival of B cells (Hombach *et al.*, 1990, Molecular components of the B-cell antigen receptor complex of the IgM class, *Nature* 343:760-762), and inefficient interspecies interactions between soluble human immunoglobulins and mouse Fc receptors that might reduce affinity selection (Rao *et al.*, 2002, Differential expression of the inhibitory IgG Fc receptor FcgammaRIIB on germinal center cells: implications for selection of high-affinity B cells, *J Immunol* 169:1859-1868) and immunoglobulin serum concentrations (Brambell *et al.*, 1964, A Theoretical Model of Gamma-

Globulin Catabolism, *Nature* 203:1352-1354; Junghans and Anderson, 1996, The protection receptor for IgG catabolism is the beta2-microglobulin-containing neonatal intestinal transport receptor, *PNAS USA* 93:5512-5516; Rao *et al.*, 2002; Hjelm *et al.*, 2006, Antibody-mediated regulation of the immune response, *Scand J Immunol* 64:177-184; Nimmerjahn and Ravetch, 2007, Fc-receptors as regulators of immunity, *Adv Immunol* 96:179-204). These deficiencies can be corrected by in situ humanization of only the variable regions of the mouse immunoglobulin loci within their natural locations at the endogenous heavy and light chain loci. This would effectively result in mice that make "reverse chimeric" (*i.e.*, human V:mouse C) antibodies which would be capable of normal interactions and selection with the mouse environment based on retaining mouse constant regions. Taking this approach, a particular version of a humanized locus can be constructed based on the complexity of the chimeric locus that is desired. Further such reverse chimeric antibodies may be readily reformatted into fully human antibodies for therapeutic purposes.

[00307] Genetically modified animals that comprise an insertion or a replacement at the endogenous immunoglobulin heavy chain locus with heterologous (*e.g.*, from another species) immunoglobulin sequences can be made in conjunction with insertions or replacements at endogenous immunoglobulin light chain loci or in conjunction with immunoglobulin light chain transgenes (*e.g.*, chimeric immunoglobulin light chain transgenes or fully human fully mouse, etc.). The species from which the heterologous immunoglobulin heavy chain sequences are derived can vary widely; as with immunoglobulin light chain sequences employed in immunoglobulin light chain sequence replacements or immunoglobulin light chain transgenes. Exemplary heterologous immunoglobulin heavy chain sequences include human sequences.

[00308] Immunoglobulin variable region nucleic acid sequences, *e.g.*, V, D, and/or J segments, are in various embodiments obtained from a human or a non-human animal. Non-human animals suitable for providing V, D, and/or J segments include, for example bony fish, cartilaginous fish such as sharks and rays, amphibians, reptiles, mammals, birds (*e.g.*, chickens). Non-human animals include, for example, mammals. Mammals include, for example, non-human primates, goats, sheep, pigs, dogs, bovine (*e.g.*, cow, bull, buffalo), deer, camels, ferrets and rodents and non-human primates (*e.g.*, chimpanzees, orangutans, gorillas, marmosets, rhesus monkeys baboons). Suitable non-human animals are selected from the rodent family including rats, mice, and hamsters. In one embodiment, the non-human animals are mice. As clear from the context, various non-human animals can be used as sources

of variable domains or variable region gene segments (*e.g.*, sharks, rays, mammals, *e.g.*, camels, rodents such as mice and rats).

[00309] According to the context, non-human animals are also used as sources of constant region sequences to be used in connection with variable sequences or segments, for example, rodent constant sequences can be used in transgenes operably linked to human or non-human variable sequences (*e.g.*, human or non-human primate variable sequences operably linked to, *e.g.*, rodent, *e.g.*, mouse or rat or hamster, constant sequences). Thus, in various embodiments, human V, D, and/or J segments are operably linked to rodent (*e.g.*, mouse or rat or hamster) constant region gene sequences. In some embodiments, the human V, D, and/or J segments (or one or more rearranged VDJ or VJ genes) are operably linked or fused to a mouse, rat, or hamster constant region gene sequence in, *e.g.*, a transgene integrated at a locus that is not an endogenous immunoglobulin locus.

[00310] In a specific embodiment, a mouse is provided that comprises a replacement of V_H, D_H, and J_H gene segments at an endogenous immunoglobulin heavy chain locus with a single human V_H, one or more D_H, and one or more J_H gene segments, wherein the single human V_H, one or more D_H, and one or more J_H gene segments are operably linked to an endogenous immunoglobulin heavy chain gene; wherein the mouse comprises a transgene at a locus other than an endogenous immunoglobulin locus, wherein the transgene comprises an unrearranged or rearranged human V_L and human J_L gene segment operably linked to a mouse or rat or human constant region. In various embodiments, the single human V_H gene segment is a polymorphic gene segment. In one embodiment, the single human V_H gene segment is a human V_H1-69 gene segment or a human V_H1-2 gene segment.

[00311] A method for an *in situ* genetic replacement of the mouse germline immunoglobulin heavy chain variable gene locus with a restricted human germline immunoglobulin heavy chain locus and replacement of the mouse germline immunoglobulin κ light chain variable gene loci with human germline immunoglobulin κ light chain loci, while maintaining the ability of the mice to generate offspring, is described. Specifically, the precise replacement of six megabases of both the mouse heavy chain and κ light chain immunoglobulin variable gene loci with human immunoglobulin heavy and κ light chain sequences, while leaving the mouse constant regions intact, is described. As a result, mice have been created that have a precise replacement of their entire germline immunoglobulin variable repertoire with human germline immunoglobulin variable sequences, while maintaining mouse constant regions. The human variable regions are linked to mouse constant regions

to form chimeric human-mouse immunoglobulin loci that rearrange and express at physiologically appropriate levels. The antibodies expressed are "reverse chimeras," *i.e.*, they comprise human variable region sequences and mouse constant region sequences.

[00312] The genetically modified mice described herein exhibit a fully functional humoral immune system and provide a plentiful source of naturally affinity-matured human immunoglobulin variable region sequences for making pharmaceutically acceptable antibodies and other antigen-binding proteins that are effective for combating pathogenic antigens, *e.g.*, viral antigens.

[00313] The engineering of human immunoglobulin sequences in the genome of a mouse, even at precise locations, *e.g.*, at the endogenous mouse immunoglobulin loci, may present certain challenges due to divergent evolution of the immunoglobulin loci between mouse and man. For example, intergenic sequences interspersed within the immunoglobulin loci are not identical between mice and humans and, in some circumstances, may not be functionally equivalent. Differences between mice and humans in their immunoglobulin loci can still result in abnormalities in humanized mice, particularly when humanizing or manipulating certain portions of endogenous mouse immunoglobulin heavy chain loci. Some modifications at mouse immunoglobulin heavy chain loci are deleterious. Deleterious modifications can include, for example, loss of the ability of the modified mice to mate and produce offspring. In various embodiments, engineering human immunoglobulin sequences in the genome of a mouse includes methods that maintain endogenous sequences that when absent in modified mouse strains are deleterious. Exemplary deleterious effects may include inability to propagate modified strains, loss of function of essential genes, inability to express polypeptides, *etc.* Such deleterious effects may be directly or indirectly related to the modification engineered into the genome of the mouse.

[00314] Notwithstanding the near wild-type humoral immune function observed in mice with humanized immunoglobulin loci, there are other challenges encountered when employing a direct replacement of immunoglobulin sequences that is not encountered in some approaches that employ randomly integrated transgenes. Differences in the genetic composition of the immunoglobulin loci between mice and humans has lead to the discovery of sequences beneficial for the propagation of mice with replaced immunoglobulin gene segments. Specifically, mouse ADAM genes located within the endogenous immunoglobulin locus are optimally present in mice with replaced immunoglobulin loci, due to their role in fertility.

[00315] A precise, *in situ* replacement of six megabases of the variable regions of

the mouse heavy chain immunoglobulin loci (V_H-D_H-J_H) with a restricted human immunoglobulin heavy chain locus was performed, while leaving the flanking mouse sequences intact and functional within the hybrid loci, including all mouse constant chain genes and locus transcriptional control regions (FIG. 1 and FIG. 8). Further engineering steps were performed to maintain mouse sequences that confer on the mouse the ability to mate and produce offspring in a manner comparable to a wild-type mouse (FIG. 9 and FIG. 10). Specifically, a single human V_H, 27 D_H, and six J_H gene segments and mouse ADAM6 genes were introduced through chimeric BAC targeting vectors into mouse ES cells using VELOCIGENE® genetic engineering 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, *Nat Biotechnol* 21:652-659).

Mice With Restricted Immunoglobulin Heavy Chain Variable Gene Segments

[00316] Non-human animals comprising immunoglobulin loci that comprise a restricted number of V_H genes, and one or more D genes and one or more J genes, are provided, as are methods of making and using them. When immunized with an antigen of interest, the non-human animals generate B cell populations with antibody variable regions derived only from the restricted, pre-selected V_H gene or set of V_H genes (*e.g.*, a pre-selected V_H gene and variants thereof). In various embodiments, non-human animals are provided that generate B cell populations that express human antibody variable domains that are human heavy chain variable domains, along with cognate human light chain variable domains. In various embodiments, the non-human animals rearrange human heavy chain variable gene segments and human light chain variable gene segments from modified endogenous mouse immunoglobulin loci that comprise a replacement or insertion of the non-human unrearranged variable region sequences with human unrearranged variable region sequences.

[00317] Early work on the organization, structure, and function of the immunoglobulin genes was done in part on mice with disabled endogenous loci and engineered to have transgenic loci (randomly placed) with partial human immunoglobulin genes, *e.g.*, a partial repertoire of human heavy chain genes linked with a human constant gene, randomly inserted into the genome, in the presence or absence of a human light chain transgene. Although these mice were somewhat less than optimal for making useful high affinity antibodies, they facilitated certain functional analyses of immunoglobulin loci. Some of these mice had as few as two or three, or even just a single, heavy chain variable gene.

[00318] Mice that express fully human immunoglobulin heavy chains derived from a single human V_H5-51 gene and 10 human D_H genes and six human J_H genes, with human μ and γ1 constant genes, on a randomly inserted transgene (and disabled endogenous immunoglobulin loci) have been reported (Xu and Davis, 2000, Diversity in the CDR3 Region of V_H Is Sufficient for Most Antibody Specificities, *Immunity* 13:37-45). The fully human immunoglobulin heavy chains of these mice are mostly expressed with one of just two fully mouse λ light chains derived from the endogenous mouse λ light chain locus (Vλ1-Jλ1 or Vλ2-Jλ2 only), and can express no κ light chain (the mice are $Ig\kappa^{-/-}$). These mice exhibit severely abnormal dysfunction in B cell development and antibody expression. B cell numbers are reportedly 5-10% of wild-type, IgM levels 5-10% of wild-type, and IgG1 levels are only 0.1-1% of wild-type. The observed IgM repertoire revealed highly restricted junctional diversity. The fully human heavy chains display largely identical CDR3 length across antigens, the same J_H (J_H2) usage across antigens, and an initial junctional Q residue, thus reflecting a certain lack of CDR3 diversity. The fully mouse λ light chains nearly all had a W96L substitution in Jλ1 as initial junctional residue. The mice are reportedly unable to generate any antibodies against bacterial polysaccharide. Because the human variable domains couple with mouse light chains, the utility of the human variable regions is highly limited.

[00319] Other mice that have just a single human V_H3-23 gene, human D_H and J_H genes, and mouse light chain genes have been reported, but they exhibit a limited diversity (and thus a limited usefulness) due in part to mispairing potential between human V_H and mouse V_L domains (see, *e.g.*, Mageed *et al.*, 2001, Rearrangement of the human heavy chain variable region gene V3-23 in transgenic mice generates antibodies reactive with a range of antigens on the basis of V_HCDR3 and residues intrinsic to the heavy chain variable region, *Clin. Exp. Immunol.* 123:1-5). Similarly, mice that bear two V_H genes (3-23 and 6-1) along with human D_H and J_H genes in a transgene containing the human μ constant gene (Bruggemann *et al.*, 1991, Human antibody production in transgenic mice: expression from 100kb of the human IgH locus, *Eur. J. Immmunol.* 21:1323-1326) and express them in human IgM chains with mouse light chains may exhibit a repertoire limited by mispairing (Mackworth-Young *et al.*, 2003, The role of antigen in the selection of the human V3-23 immunoglobulin heavy chain variable region gene, *Clin. Exp. Immunol.* 134:420-425).

[00320] Other transgenic mice that express V_H-restricted fully human heavy chains from a human transgene randomly inserted in the genome, with a limited human λ repertoire expressed from a fully human randomly inserted transgene, have

also been reported (see, *e.g.*, Taylor *et al.*, 1992, A transgenic mouse that expresses a diversity of human sequence heavy and light chain immunoglobulins, *Nucleic Acids Res.* 20(23):6287-6295; Wagner *et al.*, 1994, Antibodies generated form human immunoglobulin miniloci in transgenic mice, *Nucleic Acids Res.* 22(8):1389-1393). However, transgenic mice that express fully human antibodies from transgenes randomly integrated into the mouse genome, and that comprise damaged endogenous loci, are known to exhibit substantial differences in immune response as compared with wild-type mice that affect the diversity of the antibody variable domains obtainable from such mice.

[00321] Useful non-human animals that generate a diverse population of B cells that express human antibody variable domains from a restricted V_H gene repertoire and one or more D genes and one or more J genes will be capable of generating, preferably in some embodiments, repertoires of rearranged variable region genes that will be sufficiently diverse. In various embodiments, diversity includes junctional diversity, somatic hypermutation, and polymorphic diversity in V_H gene sequence (for embodiments where V_H genes are present in polymorphic forms). Combinatorial diversity occurs in the pairing of the V_H gene with one of a plurality of cognate human light chain variable domains (which, in various embodiments, comprise junctional diversity and/or somatic hypermutations).

[00322] Non-human animals comprising a restricted human V_H gene repertoire and a complete or substantially complete human V_L gene repertoire will in various embodiments generate populations of B cells that reflect the various sources of diversity, such as junctional diversity (*e.g.*, VDJ, VJ joining, P additions, N additions), combinatorial diversity (*e.g.*, cognate V_H-restricted human heavy, human light), and somatic hypermutations. In embodiments comprising a restriction of the V_H repertoire to one human V_H gene, the one human V_H gene can be present in two or more variants. In various embodiments, the presence of two or more polymorphic forms of a V_H gene will enrich the diversity of the variable domains of the B cell population.

[00323] Variations in the germline sequences of gene segments (*e.g.*, V genes) contribute to the diversity of the antibody response in humans. The relative contribution to diversity due to V gene sequence differences varies among V genes. The degree of polymorphism varies across gene families, and is reflected in a plurality of haplotypes (stretches of sequence with coinherited polymorphisms) capable of generating further diversity as observed in V_H haplotype differences between related and unrelated individuals in the human population (see, *e.g.*, Souroujon *et al.*, 1989, Polymorphisms in Human H Chain V Region Genes from the

V_HIII Gene Family, *J. Immunol.* 143(2):706-711). Some have suggested, based on data from particularly polymorphic human V_H gene families, that haplotype diversity in the germline is a major contributor to V_H gene heterogeneity in the human population, which is reflected in the large diversity of different germline V_H genes across the human population (see, Sasso *et al.*, 1990, Prevalence and Polymorphism of Human V_H3 Genes, *J. Immunol.* 145(8):2751-2757).

[00324] Although the human population displays a large diversity of haplotypes with respect to the V_H gene repertoire due to widespread polymorphism, certain polymorphisms are reflected in prevalent (*i.e.*, conserved) alleles observed in the human population (Sasso *et al.*, 1990). V_H polymorphism can be described in two principle forms. The first is variation arising from allelic variation associated with differences among the nucleotide sequence between alleles of the same gene segment. The second arises from the numerous duplications, insertions, and/or deletions that have occurred at the immunoglobulin heavy chain locus. This has resulted in the unique situation in which V_H genes derived by duplication from identical genes differ from their respective alleles by one or more nucleotide substitutions. This also directly influences the copy number of V_H genes at the heavy chain locus.

[00325] Polymorphic alleles of the human immunoglobulin heavy chain variable gene segments (V_H genes) have largely been the result of insertion/deletion of gene segments and single nucleotide differences within coding regions, both of which have the potential to have functional consequences on the immunoglobulin molecule. Table 1 sets forth the functional V_H genes listed by human V_H gene family and the number of identified alleles for each V_H gene in the human immunoglobulin heavy chain locus. There are some findings to suggest that polymorphic V_H genes have been implicated in susceptibility to certain diseases such as, for example, rheumatoid arthritis, whereas in other cases a linkage between V_H and disease has been less clear. This ambiguity has been attributed to the copy number and presence of various alleles in different human populations. In fact, several human V_H genes demonstrate copy number variation (*e.g.*, V_H1-2, V_H1-69, V_H2-26, V_H2-70, and V_H3-23). In various embodiments, humanized mice as described herein with restricted V_H repertoires comprise multiple polymorphic variants of an individual V_H family member (*e.g.*, two or more polymorphic variants of V_H1-2, V_H1-69, V_H2-26, V_H2-70, or V_H3-23, replacing all or substantially all functional mouse V_H segments at an endogenous mouse locus). In a specific embodiment, the two or more polymorphic variants of mice described herein are in number up to and including the number indicated for the

corresponding V_H family member in Table 1 (*e.g.*, for V_H1-69, 13 variants; for V_H1-2, five variants; *etc.*).

[00326] Commonly observed variants of particular human V_H genes are known in the art. For example, one of the most complex polymorphisms in the V_H locus belongs to the V_H1-69 gene. The human V_H1-69 gene has 13 reported alleles (Sasso *et al.*, 1993, A fetally expressed immunoglobulin V_H1 gene belongs to a complex set of alleles, *Journal of Clinical Investigation* 91:2358-2367; Sasso *et al.*, 1996, Expression of the immunoglobulin V_H gene 51p1 is proportional to its germline gene copy number, *Journal of Clinical Investigation* 97(9):2074-2080) and exists in at least three haplotypes that carry duplications of the V_H1-69 gene, which results in multiple copies of the V_H gene at a given locus. These polymorphic alleles include differences in the complementarity determining regions (CDRs), which may dramatically influence antigen specificity. Table 2 sets for the reported alleles for human V_H1-69 and human V_H1-2 genes and the SEQ ID NOs for the DNA and protein sequences of the mature heavy chain variable regions.

[00327] Representative genomic DNA and full-length protein sequences of a V_H1-69 gene are set forth in SEQ ID NO: 4 and SEQ ID NO: 5, respectively. FIG. 5 and FIG. 6 set forth DNA and protein alignments of thirteen reported V_H1-69 alleles, respectively. Representative DNA and protein sequences of a V_H1-2 gene are set forth in SEQ ID NO: 63 and SEQ ID NO: 64, respectively. FIG. 8 and FIG. 9 set forth DNA and protein alignments of five reported V_H1-2 alleles, respectively. FIG. 7 and FIG. 10 set forth a percent identity/similarity matrix for aligned protein sequences corresponding to thirteen-reported human V_H1-69 and five reported human V_H1-2 alleles, respectively. In various embodiments, the modified locus of the invention comprises a V_H gene selected from Table 1, present in two or more copy number, wherein the copy number includes up to and including the number of alleles shown in Table 1. In one embodiment, the modified locus of the invention comprises a V_H1-69 or V_H1-2 gene selected from Table 2, present in two or more copy number, wherein the copy number includes up to and including the number of alleles shown in Table 1.

Table 1

V_H Family	V_H Gene	Alleles
V_H Family 1	1-2	5
	1-3	2
	1-8	2
	1-18	3
	1-24	1
	1-45	3
	1-46	3
	1-58	2
	1-69	13
V_H Family 2	2-5	10
	2-26	1
	2-70	13
V_H Family 3	3-7	3
	3-9	2
	3-11	4
	3-13	4
	3-15	8
	3-16	2
	3-20	1
	3-21	4
	3-23	5
	3-30	19
	3-30-3	2
	3-30-5	1
	3-33	6
	3-35	1
	3-38	2
	3-43	2
	3-48	4
	3-49	5
	3-53	4
	3-64	5
	3-66	4
	3-72	2
	3-73	2
	3-74	3
V_H Family 4	4-4	7
	4-28	6
	4-30-1	1
	4-30-2	5

	4-30-4	6
	4-31	10
	4-34	13
	4-39	7
	4-59	10
	4-61	8
V_H Family 5	5-51	5
V_H Family 6	6-1	2
V_H Family 7	7-4-1	5
	7-81	1

Table 2

IgHV1-69 Allele	Accession number	SEQ ID NO: (DNA/Protein)
IgHV1-69*01	L22582	37/38
IgHV1-69*02	Z27506	39/40
IgHV1-69*03	X92340	41/42
IgHV1-69*04	M83132	43/44
IgHV1-69*05	X67905	45/46
IgHV1-69*06	L22583	47/48
IgHV1-69*07	Z29978	49/50
IgHV1-69*08	Z14309	51/52
IgHV1-69*09	Z14307	53/54
IgHV1-69*10	Z14300	55/56
IgHV1-69*11	Z14296	57/58
IgHV1-69*12	Z14301	59/60
IgHV1-69*13	Z14214	61/62

IgHV1-2 Allele	Accession number	SEQ ID NO: (DNA/Protein)
IgHV1-2*01	X07448	63/64
IgHV1-2*02	X62106	65/66
IgHV1-2*03	X92208	67/68
IgHV1-2*04	Z12310	69/70
IgHV1-2*05	HM855674	71/72

Antigen-Dependent Heavy Chain Variable Gene Usage

[00328] Antigen-dependent preferential usage of V_H genes can be exploited in the development of human therapeutics targeting clinically significant antigens. The ability to generate a repertoire of antibody variable domains using a particular V_H

gene can provide a significant advantage in the search for high-affinity antibody variable domains to use in human therapeutics. Studies on naive mouse and human V_H gene usage in antibody variable domains reveal that most heavy chain variable domains are not derived from any particularly single or dominantly used V_H gene. On the other hand, studies of antibody response to certain antigens reveal that in some cases a particular antibody response displays a biased usage of a particular V_H gene in the B cell repertoire following immunization.

[00329] Although the human V_H repertoire is quite diverse, by some estimates the expected frequency of usage of any given V_H gene, assuming random selection of V_H genes, is about 2% (Brezinschek *et al.,* 1995, Analysis of the Heavy Chain Repertoire of Human Peripheral B Cells Using Single-Cell Polymerase Chain Reaction, *J. Immunol.* 155:190-202). But V_H usage in peripheral B cells in humans is skewed. In one study, functional V gene abundance followed the pattern $V_H3 > V_H4 > V_H1 > V_H2 > V_H5 > V_H6$ (Davidkova *et al.,* 1997, Selective Usage of V_H Genes in Adult Human Lymphocyte Repertoires, *Scand. J. Immunol.* 45:62-73). One early study estimated that V_H3 family usage frequency was about 0.65, whereas V_H1 family usage frequency was about 0.15; these and other observations suggest that the germline complexity of the human V_H repertoire is not precisely reflected in the peripheral B cell compartment in humans that have a normal germline V_H repertoire, a situation that is similar to that observed in the mouse--*i.e.*, V_H gene expression is non-stochastic (Zouali and These, 1991, Probing V_H Gene-Family Utilization in Human Peripheral B Cells by *In Situ* Hybridization, *J. Immunol.* 146(8):2855-2864). According to one report, V_H gene usage in humans, from greatest to least, is $V_H3 > V_H4 > V_H1 > V_H5 > V_H2 > V_H6$; rearrangements in peripheral B cells reveal that V_H3 family usage is higher than to be expected based on the relative number of germline V_H3 genes (Brezinschek *et al.,* 1995). According to another report V_H usage in humans follows the pattern $V_H3 > V_H5 > V_H2 > V_H1 > V_H4 > V_H6$, based on analysis of pokeweed mitogen-activated peripheral small immunocompetent B cells (Davidkova *et al.,* 1997, Selective Usage of V_H Genes in Adult Human B Lymphocyte Repertoires, *Scand. J. Immunol.* 45:62-73). One report asserts that among the most frequently used V_H3 family members are 3-23, 3-30 and 3-54 (Brezinschek *et al.,* 1995). In the V_H4 family, member 4-59 and 4-4b were found relatively more frequently (*Id.*), as well as 4-39 and 4-34 (Brezinscheck *et al.,* 1997, Analysis of the Human V_H Gene Repertoire, *J. Clin. Invest.* 99(10):2488-2501). Others postulate that the activated heavy chain repertoire is skewed in favor of high V_H5 expression and lower V_H3 expression (Van Dijk-Hard and Lundkvist, 2002, Long-term kinetics of adult human antibody repertoires, *Immunology* 107:136-144). Other studies assert

that the most commonly used V_H gene in the adult human repertoire is V_H4-59, followed by V_H3-23 and V_H3-48 (Arnaout *et al.*, 2001, High-Resolution Description of Antibody Heavy-Chain Repertoires in Humans, *PLoS ONE* 6(8):108). Although usage studies are based on relatively small sample numbers and thus exhibit high variance, taken together the studies suggest that V gene expression is not purely stochastic. Indeed, studies with particular antigens have established that—in certain cases—the deck is firmly stacked against certain usages and in favor of others.

[00330] Over time, it became apparent that the observed repertoire of human heavy chain variable domains generated in response to certain antigens is highly restricted. Some antigens are associated almost exclusively with neutralizing antibodies having only certain particular V_H genes, in the sense that effective neutralizing antibodies are derived from essentially only one V_H gene. Such is the case for a number of clinically important human pathogens.

[00331] V_H1-69-derived heavy chains have been observed in a variety of antigen-specific antibody repertoires of therapeutic significance. For instance, V_H1-69 was frequently observed in heavy chain transcripts of an IgE repertoire of peripheral blood lymphocytes in young children with atopic disease (Bando *et al.*, 2004, Characterization of $V_H\varepsilon$ gene expressed in PBL from children with atopic diseases: detection of homologous V_H1-69 derived transcripts from three unrelated patients, *Immunology Letters* 94:99-106). V_H1-69-derived heavy chains with a high degree of somatic hypermutation also occur in B cell lymphomas (Perez *et al.*, 2009, Primary cutaneous B-cell lymphoma is associated with somatically hypermutated immunoglobulin variable genes and frequent use of V_H1-69 and V_H4-59 segments, *British Journal of Dermatology* 162:611-618), whereas some V_H1-69-derived heavy chains with essentially germline sequences (*i.e.*, little to no somatic hypermutation) have been observed among autoantibodies in patients with blood disorders (Pos *et al.*, 2008, V_H1-69 germline encoded antibodies directed towards ADAMTS13 in patients with acquired thrombotic thrombocytopenic purpura, *Journal of Thrombosis and Haemostasis* 7:421-428).

[00332] Further, neutralizing antibodies against viral antigens such as HIV, influenza and hepatitis C (HCV) have been found to utilize germline and/or somatically mutated V_H1-69-derived sequences (Miklos *et al.*, 2000, Salivary gland mucosa-associated lymphoid tissue lymphoma immunoglobulin V_H genes show frequent use of *V1-69* with distinctive CDR3 features, Blood 95(12):3878-3884; Kunert *et al.*, 2004, Characterization of molecular features, antigen-binding, and *in vitro* properties of IgG and IgM variants of 4E10, an anti-HIV type I neutralizing monoclonal antibody, *Aids Research and Human Retroviruses* 20(7):755-762; Chan

et al., 2001, V_H1-69 gene is preferentially used by hepatitis C virus-associated B cell lymphomas and by normal B cells responding to the E2 viral antigen, *Blood* 97(4):1023-1026; Carbonari *et al.*, 2005, Hepatitis C virus drives the unconstrained monoclonal expansion of V_H1-69-expressing memory B cells in type II cryoglobulinemia: A model of infection-driven lymphomagenesis, *Journal of Immunology* 174:6532-6539; Wang and Palese, 2009, Universal epitopes of influenza virus hemagglutinins?, *Nature Structural & Molecular Biology* 16(3):233-234; Sui *et al.*, 2009, Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses, *Nature Structural & Molecular Biology* 16(3):265-273; Marasca *et al.*, 2001, Immunoglobulin Gene Mutations and Frequent Use of V_H1-69 and V_H4-34 Segments in Hepatitis C Virus-Positive and Hepatitis C Virus-Negative Nodal Marginal Zone B-Cell Lymphoma, *Am. J. Pathol.* 159(1):253-261).

[00333] V_H usage bias is also observed in the humoral immune response to *Haemophilus influenzae* type b (Hib PS) in humans. Studies suggest that the V_HIII family (the V_HIIIb subfamily in particular, V_H9.1) exclusively characterizes the human humoral response to Hib PS, with diverse D and J genes (Adderson *et al.*, 1991, Restricted Ig H Chain V Gene Usage in the Human Antibody Response to *Haemophilus influenzae* Type b Capsular Polysaccharide, *J. Immunol.* 147(5):1667-1674; Adderson *et al.*, 1993, Restricted Immunoglobulin V_H Usage and VDJ Combinations in the Human Response to *Haemophilus influenzae* Type b Capsular Polysaccharide, *J. Clin. Invest.* 91:2734-2743). Human J_H genes also display biased usage; J_H4 and J_H6 are observed at about 38-41% in peripheral B cells in humans (Brezinschek *et al.*, 1995).

[00334] V_H usage in HIV-1-infected humans is biased against V_H3 usage and in favor of V_H1 and V_H4 gene families (Wisnewski *et al.*, 1996, Human Antibody Variable Region Gene Usage in HIV-1 Infection, *J. Acquired Immune Deficiency Syndromes & Human Retroviology* 11(1):31-38). However, cDNA analysis of bone marrow from affected patients revealed significant V_H3 usage not expressed in the functional B cell repertoire, where Fabs reflecting the V_H3 usage exhibited effective in vitro neutralization of HIV-1 (*Id.*). It might be postulated that the humoral immune response to HIV-1 infection is possibly attenuated due to the V_H restriction; modified non-human animals as described herein (not infectable by HIV-1) might thus be useful for generating neutralizing antibody domains derived from particular V_H genes present in the genetically modified animals described herein, but derived from different V_H genes than those observed in the restricted repertoire of affected humans.

[00335] Thus, the ability to generate high affinity human antibody variable domains in V_H-restricted mice, *e.g.*, (restricted, *e.g.*, to a V_H3 family member and polymorph(s) thereof) immunized with HIV-1 might provide a rich resource for designing effective HIV-1-neutralizing human therapeutics by thoroughly mining the restricted (*e.g.*, restricted to a V_H3 family member or variant(s) thereof) repertoire of such an immunized mouse.

[00336] Restriction of the human antibody response to certain pathogens may reduce the likelihood of obtaining antibody variable regions from affected humans that can serve as springboards for designing high affinity neutralizing antibodies against the pathogen. For example, the human immune response to HIV-1 infection is clonally restricted throughout HIV-1 infection and into AIDS progression (Muller *et al.*, 1993, B-cell abnormalities in AIDS: stable and clonally restricted antibody response in HIV-1 infection, *Scand. J. Immunol.* 38:327-334; Wisnewski *et al.*, 1996). Further, V_H genes are in general not present in all polymorphic forms in individuals; certain individuals in certain populations possess one variant, whereas individuals in other populations possess a different variant. Thus, the availability of a biological system that is restricted to a single V_H gene and its variants will in various embodiments provide a hitherto unexploited source of diversity for generating antibody variable regions (*e.g.*, human heavy and light cognate domains) based on a restricted V_H gene.

[00337] Genetically modified mice that express human heavy chain variable regions with restricted V_H gene segment usage are useful to generate a relatively large repertoire of junctionally diverse, combinatorially diverse, and somatically mutated high affinity human immunoglobulin heavy chain variable regions from an otherwise restricted repertoire. A restricted repertoire, in this instance, refers to a predetermined limitation in germline genes that results in the mouse being unable to form a rearranged heavy chain gene that is derived from any V gene other than a preselected V gene. In embodiments that employ a preselected V gene but not a preselected D and/or J gene, the repertoire is restricted with respect to the identity of the V gene but not the D and/or J gene. The identity of the preselected V gene (and any preselected D and/or J genes) is not limited to any particular V gene.

[00338] Designing a mouse so that it rearranges a single V_H gene (present as a single segment or a set of variants) with a variety of human D and J gene segments (e.g., D_H and J_H segments) provides an *in vivo* junctional diversity/combinatorial diversity/somatic hypermutation permutation machine that can be used to iterate mutations in resulting rearranged heavy chain variable region sequences (*e.g.*, V/D/J or V/J, as the case may be). In such a mouse, the clonal selection process operates

to select suitable variable regions that bind an antigen of interest that are based on a single preselected V_H gene (or variants thereof). Because the mouse's clonal selection components are dedicated to selection based on the single preselected V_H gene segment, background noise is largely eradicated. With judicious selection of the V_H gene segment, a relatively larger number of clonally selected, antigen-specific antibodies can be screened in a shorter period of time than with a mouse with a large diversity of V segments.

[00339] Preselecting and restricting a mouse to a single V segment provides a system for permuting V/D/J junctions at a rate that is in various embodiments higher than that observed in mice that otherwise have up to 40 or more V segments to recombine with D and J regions. Removal of other V segments frees the locus to form more V/D/J combinations for the preselected V segment than otherwise observed. The increased number of transcripts that result from the recombination of the preselected V with one of a plurality of D and one of a plurality of J segments will feed those transcripts into the clonal selection system in the form of pre-B cells, and the clonal selection system is thus dedicated to cycling B cells that express the preselected V region. In this way, more unique V regions derived from the preselected V segment can be screened by the organism than would otherwise be possible in a given amount of time.

[00340] In various aspects, mice are described that enhance the junctional diversity of V/D recombinations for the preselected V region, because all or substantially all recombinations of the immunoglobulin heavy chain variable locus will be of the preselected V segment and the D and J segments that are placed in such mice. Therefore, the mice provide a method for generating a diversity of CDR3 segments using a base, or restricted V_H gene repertoire.

Genomic Location and Function of Mouse ADAM6

[00341] Male mice that lack the ability to express any functional ADAM6 protein surprisingly exhibit a defect in the ability of the mice to mate and to generate offspring. The mice lack the ability to express a functional ADAM6 protein by virtue of a replacement of all or substantially all mouse immunoglobulin variable region gene segments with human variable region gene segments. The loss of ADAM6 function results because the ADAM6 locus is located within a region of the endogenous mouse immunoglobulin heavy chain variable region gene locus, proximal to the 3' end of the V_H gene segment locus that is upstream of the D_H gene segments. In order to breed mice that are homozygous for a replacement of all or substantially all endogenous mouse heavy chain variable sequences with a restricted

human heavy chain sequence, it is generally a cumbersome approach to set up males and females that are each homozygous for the restricted human heavy chain sequence and await a productive mating. Successful litters are low in frequency and size. Instead, males heterozygous for the restricted human heavy chain sequence have been employed to mate with females homozygous for the replacement to generate progeny that are heterozygous for the restricted human heavy chain sequence, then a homozygous mouse is bred therefrom. The inventors have determined that the likely cause of the loss in fertility in the male mice is the absence in homozygous male mice of a functional ADAM6 protein.

[00342] In various aspects, male mice that comprise a damaged (*i.e.*, nonfunctional or marginally functional) ADAM6 gene exhibit a reduction or elimination of fertility. Because in mice (and other rodents) the ADAM6 gene is located in the immunoglobulin heavy chain locus, the inventors have determined that in order to propagate mice, or create and maintain a strain of mice, that comprise a humanized immunoglobulin heavy chain locus, various modified breeding or propagation schemes are employed. The low fertility, or infertility, of male mice homozygous for a humanized immunoglobulin heavy chain variable gene locus renders maintaining such a modification in a mouse strain difficult. In various embodiments, maintaining the strain comprises avoiding infertility problems exhibited by male mice homozygous for the humanized heavy chain locus.

[00343] In one aspect, a method for maintaining a strain of mouse as described herein is provided. The strain of mouse need not comprise an ectopic ADAM6 sequence, and in various embodiments the strain of mouse is homozygous or heterozygous for a knockout (*e.g.*, a functional knockout) of ADAM6.

[00344] The mouse strain comprises a modification of an endogenous immunoglobulin heavy chain locus that results in a reduction or loss in fertility in a male mouse. In one embodiment, the modification comprises a deletion of a regulatory region and/or a coding region of an ADAM6 gene. In a specific embodiment, the modification comprises a modification of an endogenous ADAM6 gene (regulatory and/or coding region) that reduces or eliminates fertility of a male mouse that comprises the modification; in a specific embodiment, the modification reduces or eliminates fertility of a male mouse that is homozygous for the modification.

[00345] In one embodiment, the mouse strain is homozygous or heterozygous for a knockout (*e.g.*, a functional knockout) or a deletion of an ADAM6 gene.

[00346] In one embodiment, the mouse strain is maintained by isolating from a mouse that is homozygous or heterozygous for the modification a cell, and

employing the donor cell in host embryo, and gestating the host embryo and donor cell in a surrogate mother, and obtaining from the surrogate mother a progeny that comprises the genetic modification. In one embodiment, the donor cell is an ES cell. In one embodiment, the donor cell is a pluripotent cell, e.g., an induced pluripotent cell.

[00347] In one embodiment, the mouse strain is maintained by isolating from a mouse that is homozygous or heterozygous for the modification a nucleic acid sequence comprising the modification, and introducing the nucleic acid sequence into a host nucleus, and gestating a cell comprising the nucleic acid sequence and the host nucleus in a suitable animal. In one embodiment, the nucleic acid sequence is introduced into a host oocyte embryo.

[00348] In one embodiment, the mouse strain is maintained by isolating from a mouse that is homozygous or heterozygous for the modification a nucleus, and introducing the nucleus into a host cell, and gestating the nucleus and host cell in a suitable animal to obtain a progeny that is homozygous or heterozygous for the modification.

[00349] In one embodiment, the mouse strain is maintained by employing in vitro fertilization (IVF) of a female mouse (wild-type, homozygous for the modification, or heterozygous for the modification) employing a sperm from a male mouse comprising the genetic modification. In one embodiment, the male mouse is heterozygous for the genetic modification. In one embodiment, the male mouse is homozygous for the genetic modification.

[00350] In one embodiment, the mouse strain is maintained by breeding a male mouse that is heterozygous for the genetic modification with a female mouse to obtain progeny that comprises the genetic modification, identifying a male and a female progeny comprising the genetic modification, and employing a male that is heterozygous for the genetic modification in a breeding with a female that is wild-type, homozygous, or heterozygous for the genetic modification to obtain progeny comprising the genetic modification. In one embodiment, the step of breeding a male heterozygous for the genetic modification with a wild-type female, a female heterozygous for the genetic modification, or a female homozygous for the genetic modification is repeated in order to maintain the genetic modification in the mouse strain.

[00351] In one aspect, a method is provided for maintaining a mouse strain that comprises a replacement of an endogenous immunoglobulin heavy chain variable gene locus with one or more human immunoglobulin heavy chain sequences, comprising breeding the mouse strain so as to generate heterozygous male mice,

wherein the heterozygous male mice are bred to maintain the genetic modification in the strain. In a specific embodiment, the strain is not maintained by any breeding of a homozygous male with a wild-type female, or a female homozygous or heterozygous for the genetic modification.

[00352] The ADAM6 protein is a member of the A Disintegrin And Metalloprotease (ADAM) family of proteins, which is a large family of proteins having diverse functions including cell adhesion. Some members of the ADAM family are implicated in spermatogenesis and fertilization. For example, ADAM2 encodes a subunit of the protein fertilin, which is implicated in sperm-egg interactions. ADAM3, or cyritestin, appears necessary for sperm binding to the zona pellucida. The absence of either ADAM2 or ADAM3 results in infertility. It has been postulated that ADAM2, ADAM3, and ADAM6 form a complex on the surface of mouse sperm cells. The human counterpart gene (human ADAM6), normally found between human V_H gene segments V_H1-2 and V_H6-1 in the human immunoglobulin heavy chain locus, appears to be a pseudogene. In mice, there are two ADAM6 genes—ADAM6a and ADAM6b—that are found in an intergenic region between mouse V_H and D_H gene segments, and in the mouse the ADAM6a and ADAM6b genes are oriented in opposite transcriptional orientation to that of the surrounding immunoglobulin gene segments. In mice, a functional ADAM6 locus is apparently required for normal fertilization. A functional ADAM6 locus or sequence, then, refers to an ADAM6 locus or sequence that can complement, or rescue, the drastically reduced fertilization exhibited in male mice with missing or nonfunctional endogenous ADAM6 loci.

[00353] The position of the intergenic sequence in mice that encodes ADAM6a and ADAM6b renders the intergenic sequence susceptible to modification when modifying an endogenous mouse heavy chain. When V_H gene segments are deleted or replaced, or when D_H gene segments are deleted or replaced, there is a high probability that a resulting mouse will exhibit a severe deficit in fertility. In order to compensate for the deficit, the mouse is modified to include a nucleotide sequence that encodes a protein that will complement the loss in ADAM6 activity due to a modification of the endogenous mouse ADAM6 locus. In various embodiments, the complementing nucleotide sequence is one that encodes a mouse ADAM6a, a mouse ADAM6b, or a homolog or ortholog or functional fragment thereof that rescues the fertility deficit. Alternatively, suitable methods to preserve the endogenous ADAM6 locus can be employed, while rendering the endogenous immunoglobulin heavy chain sequences flanking the mouse ADAM6 locus incapable of rearranging to encode a functional endogenous heavy chain variable region. Exemplary alternative methods include manipulation of large portions of mouse

chromosomes that position the endogenous immunoglobulin heavy chain variable region loci in such a way that they are incapable of rearranging to encode a functional heavy chain variable region that is operably linked to an endogenous heavy chain constant gene. In various embodiments, the methods include inversions and/or translocations of mouse chromosomal fragments containing endogenous immunoglobulin heavy chain gene segments.

[00354] The nucleotide sequence that rescues fertility can be placed at any suitable position. It can be placed in the intergenic region, or in any suitable position in the genome (*i.e.*, ectopically). In one embodiment, the nucleotide sequence can be introduced into a transgene that randomly integrates into the mouse genome. In one embodiment, the sequence can be maintained episomally, that is, on a separate nucleic acid rather than on a mouse chromosome. Suitable positions include positions that are transcriptionally permissive or active, *e.g.*, a ROSA26 locus (Zambrowicz *et al.*, 1997, *PNAS USA* 94:3789-3794), a BT-5 locus (Michael *et al.*, 1999, *Mech. Dev.* 85:35-47), or an Oct4 locus (Wallace *et al.*, 2000, *Nucleic Acids Res.* 28:1455-1464). Targeting nucleotide sequences to transcriptionally active loci are described, *e.g.*, in US 7,473,557.

[00355] Alternatively, the nucleotide sequence that rescues fertility can be coupled with an inducible promoter so as to facilitate optimal expression in the appropriate cells and/or tissues, *e.g.*, reproductive tissues. Exemplary inducible promoters include promoters activated by physical (*e.g.*, heat shock promoter) and/or chemical means (*e.g.*, IPTG or Tetracycline).

[00356] Further, expression of the nucleotide sequence can be linked to other genes so as to achieve expression at specific stages of development or within specific tissues. Such expression can be achieved by placing the nucleotide sequence in operable linkage with the promoter of a gene expressed at a specific stage of development. For example, immunoglobulin sequences from one species engineered into the genome of a host species are place in operable linkage with a promoter sequence of a CD19 gene (a B cell specific gene) from the host species. B cell-specific expression at precise developmental stages when immunoglobulins are expressed is achieved.

[00357] Yet another method to achieve robust expression of an inserted nucleotide sequence is to employ a constitutive promoter. Exemplary constitutive promoters include SV40, CMV, UBC, EF1A, PGK and CAGG. In a similar fashion, the desired nucleotide sequence is placed in operable linkage with a selected constitutive promoter, which provides high level of expression of the protein(s) encoded by the nucleotide sequence.

[00358] The term "ectopic" is intended to include a displacement, or a placement at a position that is not normally encountered in nature (*e.g.*, placement of a nucleic acid sequence at a position that is not the same position as the nucleic acid sequence is found in a wild-type mouse). The term, in various embodiments, is used in the sense of its object being out of its normal, or proper, position. For example, the phrase "an ectopic nucleotide sequence encoding..." refers to a nucleotide sequence that appears at a position at which it is not normally encountered in the mouse. For example, in the case of an ectopic nucleotide sequence encoding a mouse ADAM6 protein (or an ortholog or homolog or fragment thereof that provides the same or similar fertility benefit on male mice), the sequence can be placed at a different position in the mouse's genome than is normally found in a wild-type mouse. In such cases, novel sequence junctions of mouse sequence will be created by placing the sequence at a different position in the mouse's genome than in a wild-type mouse. A functional homolog or ortholog of mouse ADAM6 is a sequence that confers a rescue of fertility loss (*e.g.*, loss of the ability of a male mouse to generate offspring by mating) that is observed in an ADAM6$^{-/-}$ mouse. Functional homologs or orthologs include proteins that have at least about 89% identity or more, e.g., up to 99% identity, to the amino acid sequence of ADAM6a and/or to the amino acid sequence of ADAM6b, and that can complement, or rescue ability to successfully mate, of a mouse that has a genotype that includes a deletion or knockout of ADAM6a and/or ADAM6b.

[00359] The ectopic position can be anywhere (*e.g.*, as with random insertion of a transgene containing a mouse ADAM6 sequence), or can be, *e.g.*, at a position that approximates (but is not precisely the same as) its location in a wild-type mouse (*e.g.*, in a modified endogenous mouse immunoglobulin locus, but either upstream or downstream of its natural position, *e.g.*, within a modified immunoglobulin locus but between different gene segments, or at a different position in a mouse V-D intergenic sequence). One example of an ectopic placement is maintaining the position normally found in wild-type mice within the endogenous immunoglobulin heavy chain locus while rendering the surrounding endogenous heavy chain gene segments in capable of rearranging to encode a functional heavy chain containing an endogenous heavy chain constant region. In this example, this may be accomplished by inversion of the chromosomal fragment containing the endogenous immunoglobulin heavy chain variable loci, *e.g.* using engineered site-specific recombination sites placed at positions flanking the variable region locus. Thus, upon recombination the endogenous heavy chain variable region loci are placed at a great distance away from the endogenous heavy chain constant region genes thereby preventing

rearrangement to encode a functional heavy chain containing an endogenous heavy chain constant region. Other exemplary methods to achieve functional silencing of the endogenous immunoglobulin heavy chain variable gene locus while maintaining a functional ADAM6 locus will apparent to persons of skill upon reading this disclosure and/or in combination with methods known in the art. With such a placement of the endogenous heavy chain locus, the endogenous ADAM6 genes are maintained and the endogenous immunoglobulin heavy chain locus is functionally silenced.

[00360] Another example of an ectopic placement is placement within a humanized immunoglobulin heavy chain locus. For example, a mouse comprising a replacement of one or more endogenous V_H gene segments with a single human V_H gene segment, wherein the replacement removes an endogenous ADAM6 sequence, can be engineered to have a mouse ADAM6 sequence located within an intergenic sequence that lies between the single human V_H gene segment and a human D_H gene segment. Another example of an ectopic placement is placement of the mouse ADAM6 sequence at a position 5' (with respect to the direction of transcription of the single human V_H gene segment) to the human V_H gene segment. A position 5' to the single human VH gene segment may be in close proximity, *e.g.*, a few hundred base pairs to a few kb, or distant, *e.g.*, several kb to hundreds of kb or even a megabase or greater, relative to the human V_H gene segment. The resulting modification would generate a (ectopic) mouse ADAM6 sequence within or contiguous, or even on the same chromosome, with a human gene sequence, and the (ectopic) placement of the mouse ADAM6 sequence within the human gene sequence can approximate the position of the mouse ADAM6 sequence (*i.e.*, within the V-D intergenic region). The resulting sequence junctions created by the joining of a (ectopic) mouse ADAM6 sequence within or adjacent to a human gene sequence (*e.g.*, an immunoglobulin gene sequence) within the germline of the mouse would be novel as compared to the same or similar position in the genome of a wild-type mouse.

[00361] In various embodiments, non-human animals are provided that lack an ADAM6 or ortholog or homolog thereof, wherein the lack renders the non-human animal infertile, or substantially reduces fertility of the non-human animal. In various embodiments, the lack of ADAM6 or ortholog or homolog thereof is due to a modification of an endogenous immunoglobulin heavy chain locus. A substantial reduction in fertility is, *e.g.*, a reduction in fertility (*e.g.*, breeding frequency, pups per litter, litters per year, *etc.*) of about 50%, 60%, 70%, 80%, 90%, or 95% or more. In various embodiments, the non-human animals are supplemented with a mouse

ADAM6 gene or ortholog or homolog or functional fragment thereof that is functional in a male of the non-human animal, wherein the supplemented ADAM6 gene or ortholog or homolog or functional fragment thereof rescues the reduction in fertility in whole or in substantial part. A rescue of fertility in substantial part is, *e.g.*, a restoration of fertility such that the non-human animal exhibits a fertility that is at least 70%, 80%, or 90% or more as compared with an unmodified (*i.e.*, an animal without a modification to the ADAM6 gene or ortholog or homolog thereof) heavy chain locus.

[00362] The sequence that confers upon the genetically modified animal (*i.e.*, the animal that lacks a functional ADAM6 or ortholog or homolog thereof, due to, *e.g.*, a modification of a immunoglobulin heavy chain locus) is, in various embodiments, selected from an ADAM6 gene or ortholog or homolog thereof. For example, in a mouse, the loss of ADAM6 function is rescued by adding, in one embodiment, a mouse ADAM6 gene. In one embodiment, the loss of ADAM6 function in the mouse is rescued by adding an ortholog or homolog of a closely related specie with respect to the mouse, *e.g.*, a rodent, *e.g.*, a mouse of a different strain or species, a rat of any species, a rodent; wherein the addition of the ortholog or homolog to the mouse rescues the loss of fertility due to loss of ADAM6 function or loss of an ADAM6 gene. Orthologs and homologs from other species, in various embodiments, are selected from a phylogenetically related species and, in various embodiments, exhibit a percent identity with the endogenous ADAM6 (or ortholog) that is about 80% or more, 85% or more, 90% or more, 95% or more, 96% or more, or 97% or more; and that rescue ADAM6-related or (in a non-mouse) ADAM6 ortholog-related loss of fertility. For example, in a genetically modified male rat that lacks ADAM6 function (*e.g.*, a rat with an endogenous immunoglobulin heavy chain variable region replaced with a human immunoglobulin heavy chain variable region, or a knockout in the rat immunoglobulin heavy chain region), loss of fertility in the rat is rescued by addition of a rat ADAM6 or, in some embodiments, an ortholog of a rat ADAM6 (*e.g.*, an ADAM6 ortholog from another rat strain or species, or, in one embodiment, from a mouse).

[00363] Thus, in various embodiments, genetically modified animals that exhibit no fertility or a reduction in fertility due to modification of a nucleic acid sequence encoding an ADAM6 protein (or ortholog or homolog thereof) or a regulatory region operably linked with the nucleic acid sequence, comprise a nucleic acid sequence that complements, or restores, the loss in fertility where the nucleic acid sequence that complements or restores the loss in fertility is from a different strain of the same species or from a phylogenetically related species. In various embodiments, the

complementing nucleic acid sequence is an ADAM6 ortholog or homolog or functional fragment thereof. In various embodiments, the complementing ADAM6 ortholog or homolog or functional fragment thereof is from a non-human animal that is closely related to the genetically modified animal having the fertility defect. For example, where the genetically modified animal is a mouse of a particular strain, an ADAM6 ortholog or homolog or functional fragment thereof can be obtained from a mouse of another strain, or a mouse of a related species. In one embodiment, where the genetically modified animal comprising the fertility defect is of the order Rodentia, the ADAM6 ortholog or homolog or functional fragment thereof is from another animal of the order Rodentia. In one embodiment, the genetically modified animal comprising the fertility defect is of a suborder Myomoropha (*e.g.*, jerboas, jumping mice, mouse-like hamsters, hamsters, New World rats and mice, voles, true mice and rats, gerbils, spiny mice, crested rats, climbing mice, rock mice, white-tailed rats, malagasy rats and mice, spiny dormice, mole rats, bamboo rats, zokors), and the ADAM6 ortholog or homolog or functional fragment thereof is selected from an animal of order Rodentia, or of the suborder Myomorpha.

[00364] In one embodiment, the genetically modified animal is from the superfamily Dipodoidea, and the ADAM6 ortholog or homolog or functional fragment thereof is from the superfamily Muroidea. In one embodiment, the genetically modified animal is from the superfamily Muroidea, and the ADAM6 ortholog or homolog or functional fragment thereof is from the superfamily Dipodoidea.

[00365] In one embodiment, the genetically modified animal is a rodent. In one embodiment, the rodent is selected from the superfamily Muroidea, and the ADAM6 ortholog or homolog is from a different species within 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); and the ADAM6 ortholog or homolog is selected from a different species of the same family. In a specific embodiment, the genetically modified rodent is selected from a true mouse or rat (family Muridae), and the ADAM6 ortholog or homolog is from a species selected from a gerbil, spiny mouse, or crested rat. In one embodiment, the genetically modified mouse is from a member of the family Muridae, and the ADAM6 ortholog or homolog is from a different species of the family Muridae. In a specific embodiment, the genetically modified rodent is a

mouse of the family Muridae, and the ADAM6 ortholog or homolog is from a rat, gerbil, spiny mouse, or crested rat of the family Muridae.

[00366] In various embodiments, one or more rodent ADAM6 orthologs or homologs or functional fragments thereof of a rodent in a family restores fertility to a genetically modified rodent of the same family that lacks an ADAM6 ortholog or homolog (*e.g.*, Cricetidae (*e.g.*, hamsters, New World rats and mice, voles); Muridae (*e.g.*, true mice and rats, gerbils, spiny mice, crested rats)).

[00367] In various embodiments, ADAM6 orthologs, homologs, and fragments thereof are assessed for functionality by ascertaining whether the ortholog, homolog, or fragment restores fertility to a genetically modified male non-human animal that lacks ADAM6 activity (*e.g.*, a rodent, *e.g.*, a mouse or rat, that comprises a knockout of ADAM6 or its ortholog). In various embodiments, functionality is defined as the ability of a sperm of a genetically modified animal lacking an endogenous ADAM6 or ortholog or homolog thereof to migrate an oviduct and fertilize an ovum of the same specie of genetically modified animal.

[00368] In various aspects, mice that comprise deletions or replacements of the endogenous heavy chain variable region locus or portions thereof can be made that contain an ectopic nucleotide sequence that encodes a protein that confers similar fertility benefits to mouse ADAM6 (*e.g.*, an ortholog or a homolog or a fragment thereof that is functional in a male mouse). The ectopic nucleotide sequence can include a nucleotide sequence that encodes a protein that is an ADAM6 homolog or ortholog (or fragment thereof) of a different mouse strain or a different species, *e.g.*, a different rodent species, and that confers a benefit in fertility, *e.g.*, increased number of litters over a specified time period, and/or increased number of pups per litter, and/or the ability of a sperm cell of a male mouse to traverse through a mouse oviduct to fertilize a mouse egg.

[00369] In one embodiment, the ADAM6 is a homolog or ortholog that is at least 89% to 99% identical to a mouse ADAM6 protein (*e.g.*, at least 89% to 99% identical to mouse ADAM6a or mouse ADAM6b). In one embodiment, the ectopic nucleotide sequence encodes one or more proteins independently selected from a protein at least 89% identical to mouse ADAM6a, a protein at least 89% identical to mouse ADAM6b, and a combination thereof. In one embodiment, the homolog or ortholog is a rat, hamster, mouse, or guinea pig protein that is or is modified to be about 89% or more identical to mouse ADAM6a and/or mouse ADAM6b. In one embodiment, the homolog or ortholog is or is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a mouse ADAM6a and/or mouse ADAM6b.

Ectopic ADAM6 in Humanized Heavy Chain Mice

[00370] Developments in gene targeting, *e.g.*, the development of bacterial artificial chromosomes (BACs), now enable the recombination of relatively large genomic fragments. BAC engineering has allowed for the ability to make large deletions, and large insertions, into mouse ES cells.

[00371] Mice that make human antibodies have been available for some time now. Although they represent an important advance in the development of human therapeutic antibodies, these mice display a number of significant abnormalities that limit their usefulness. For example, they display compromised B cell development. The compromised development may be due to a variety of differences between the transgenic mice and wild-type mice.

[00372] Human antibodies might not optimally interact with mouse pre B cell or B cell receptors on the surface of mouse cells that signal for maturation, proliferation, or survival during clonal selection. Fully human antibodies might not optimally interact with a mouse Fc receptor system; mice express Fc receptors that do not display a one-to-one correspondence with human Fc receptors. Finally, various mice that make fully human antibodies do not include all genuine mouse sequences, *e.g.*, downstream enhancer elements and other locus control elements, which may be required for wild-type B cell development.

[00373] Mice that make fully human antibodies generally comprise endogenous immunoglobulin loci that are disabled in some way, and human transgenes that comprise variable and constant immunoglobulin gene segments are introduced into a random location in the mouse genome. As long as the endogenous locus is sufficiently disabled so as not to rearrange gene segments to form a functional immunoglobulin gene, the goal of making fully human antibodies in such a mouse can be achieved—albeit with compromised B cell development.

[00374] Although compelled to make fully human antibodies from the human transgene locus, generating human antibodies in a mouse is apparently an unfavored process. In some mice, the process is so unfavored as to result in formation of chimeric human variable/mouse constant heavy chains (but not light chains) through the mechanism of trans-switching. By this mechanism, transcripts that encode fully human antibodies undergo isotype switching in trans from the human isotype to a mouse isotype. The process is in trans, because the fully human transgene is located apart from the endogenous locus that retains an undamaged copy of a mouse heavy chain constant region gene. Although in such mice trans-switching is readily apparent the phenomenon is still insufficient to rescue B cell development, which remains frankly impaired. In any event, trans-switched

antibodies made in such mice retain fully human light chains, since the phenomenon of trans-switching apparently does not occur with respect to light chains; trans-switching presumably relies on switch sequences in endogenous loci used (albeit differently) in normal isotype switching in cis. Thus, even when mice engineered to make fully human antibodies select a trans-switching mechanism to make antibodies with mouse constant regions, the strategy is still insufficient to rescue normal B cell development.

[00375] A primary concern in making antibody-based human therapeutics, *e.g.*, anti-pathogen antibodies, is identifying useful variable domains that specifically recognize particular epitopes and bind them with a desirable affinity, usually—but not always—with high affinity. Mice as described herein, which contain a precise replacement of mouse immunoglobulin heavy chain variable regions with a restricted number of human immunoglobulin heavy chain variable gene segments at the endogenous mouse loci, display near wild-type B cell development and the variable regions generated in response to immunization are fully human, wherein the heavy chains are derived from a single human V_H gene segment. Thus, such mice provide a platform to generate a panel of heavy chain complementary determining regions (CDRs) that are specifically directed to bind a given antigen, *e.g.*, a pathogenic virus.

[00376] Mice as described herein contain a precise, large-scale replacement of germline variable gene loci of mouse immunoglobulin heavy chain (IgH) with a restricted human immunoglobulin heavy chain variable locus, and immunoglobulin light chain (*e.g.*, κ light chain, Igκ) with an equivalent human immunoglobulin κ light chain variable gene locus, at the endogenous loci. This precise replacement results in a mouse with hybrid immunoglobulin loci that make heavy and light chains that have a human variable regions and a mouse constant region. The precise replacement of mouse V_H-D_H-J_H and Vκ-Jκ segments leave flanking mouse sequences intact and functional at the hybrid immunoglobulin loci. The humoral immune system of the mouse functions like that of a wild-type mouse. B cell development is unhindered in any significant respect and a somatically mutated panel of human heavy chain CDRs is generated in the mouse upon antigen challenge.

[00377] Mice as described herein are possible because immunoglobulin gene segments for heavy and κ light chains rearrange similarly in humans and mice, which is not to say that their loci are the same or even nearly so—clearly they are not. However, the loci are similar enough that humanization of the heavy chain variable gene locus can be accomplished by replacing about three million base pairs of

contiguous mouse sequence that contains all the V_H, D_H, and J_H gene segments with a contiguous human genomic sequence containing a restricted human heavy chain locus.

[00378] In some embodiments, further replacement of certain mouse constant region gene sequences with human gene sequences (*e.g.*, replacement of mouse C_H1 sequence with human C_H1 sequence, and replacement of mouse C_L sequence with human C_L sequence) results in mice with hybrid immunoglobulin loci that make antibodies that have human variable regions and partly human constant regions, suitable for, *e.g.*, making fully human antibody fragments, *e.g.*, fully human Fab's. Mice with hybrid immunoglobulin loci exhibit normal variable gene segment rearrangement, normal somatic hypermutation frequencies, and normal class switching. These mice exhibit a humoral immune system that is indistinguishable from wild type mice, and display normal cell populations at all stages of B cell development and normal lymphoid organ structures—even where the mice lack a full repertoire of human variable region gene segments. Immunizing these mice results in robust humoral responses that display a wide diversity of heavy chain CDRs and light chain variable gene segment usage.

[00379] The precise replacement of mouse germline variable region gene segments allows for making mice that have partly human immunoglobulin loci. Because the partly human immunoglobulin loci rearrange, hypermutate, and class switch normally, the partly human immunoglobulin loci generate antibodies in a mouse that comprise human variable regions. Nucleotide sequences that encode the variable regions can be identified and cloned, then fused (*e.g.*, in an in vitro system) with any sequences of choice, *e.g.*, any immunoglobulin isotype suitable for a particular use, resulting in an antibody or antigen-binding protein derived wholly from human sequences.

[00380] Large-scale humanization by recombineering methods were used to modify mouse embryonic stem (ES) cells to precisely replace up to three megabases of the mouse heavy chain immunoglobulin locus that included essentially all of the mouse V_H, D_H, and J_H gene segments with a human genomic sequence containing a restricted human heavy chain locus including a single human V_H gene segment, 27 human D_H gene segments, and six human J_H gene segments. Up to a one-half megabase segment of the human genome comprising one of two repeats encoding essentially all human $V\kappa$ and $J\kappa$ gene segments was used to replace a three megabase segment of the mouse immunoglobulin κ light chain locus containing essentially all of the mouse $V\kappa$ and $J\kappa$ gene segments.

[00381] Mice with such replaced immunoglobulin loci can comprise a disruption or deletion of the endogenous mouse ADAM6 locus, which is normally found between the 3'-most V_H gene segment and the 5'-most D_H gene segment at the mouse immunoglobulin heavy chain locus. Disruption in this region can lead to reduction or elimination of functionality of the endogenous mouse ADAM6 locus. If one, or both, of the 3'-most V_H gene segments of the human heavy chain repertoire are used in construction of the restricted human heavy chain locus, an intergenic region containing a pseudogene that appears to be a human ADAM6 pseudogene is present between these V_H gene segments, *i.e.*, between human V_H1-2 and V_H1-6. However, male mice that comprise this human intergenic sequence exhibit a reduction in fertility (see USSN 13/404,075).

[00382] Mice are described that comprise the restricted human heavy chain and equivalent human κ light chain loci as described above, and that also comprise an ectopic nucleic acid sequence encoding a mouse ADAM6, where the mice exhibit essentially normal fertility. In one embodiment, the ectopic nucleic acid sequence comprises a mouse ADAM6a and/or a mouse ADAM6b sequence or functional fragments thereof placed between a human V_H1-69 and a human D_H1-1 at a modified endogenous heavy chain locus. In one embodiment, the ectopic nucleic acid sequence is SEQ ID NO: 77, placed between a human V_H1-69 and a human D_H1-1 at a modified endogenous heavy chain locus. The direction of transcription of the ADAM6 genes of SEQ ID NO: 77 are opposite with respect to the direction of transcription of the surrounding human gene segments. In one embodiment, the ectopic nucleic acid sequence comprises a mouse ADAM6a and/or a mouse ADAM6b sequence or functional fragments thereof placed upstream (or 5') of a human V_H1-2 gene segment at a modified endogenous heavy chain locus. In one embodiment, the ectopic nucleic acid sequence is SEQ ID NO: 73, placed upstream (or 5') of a human V_H1-2 gene segment at a modified endogenous heavy chain locus. The direction of transcription of the ADAM6 genes of SEQ ID NO: 73 are opposite with respect to the direction of transcription of the surrounding human gene segments (*e.g.* a human V_H1-2 gene segment).

[00383] Although examples herein show rescue of fertility by placing the ectopic sequence between the indicated human gene segments, skilled persons will recognize that placement of the ectopic sequence at any suitable transcriptionally-permissive locus in the mouse genome (or even extrachromosomally) will be expected to similarly rescue fertility in a male mouse.

[00384] The phenomenon of complementing a mouse that lacks a functional ADAM6 locus with an ectopic sequence that comprises a mouse ADAM6 gene or

ortholog or homolog or functional fragment thereof is a general method that is applicable to rescuing any mice with nonfunctional or minimally functional endogenous ADAM6 loci. Thus, a great many mice that comprise an ADAM6-disrupting modification of the immunoglobulin heavy chain locus can be rescued with the compositions and methods of the invention. Accordingly, the invention comprises mice with a wide variety of modifications of immunoglobulin heavy chain loci that compromise endogenous ADAM6 function. Some (non-limiting) examples are provided in this description. In addition to the mice described, the compositions and methods related to ADAM6 can be used in a great many applications, *e.g.*, when modifying a heavy chain locus in a wide variety of ways.

[00385] In one aspect, a mouse is provided that comprises an ectopic ADAM6 sequence that encodes a functional ADAM6 protein (or ortholog or homolog or functional fragment thereof), a replacement of all or substantially all mouse V_H gene segments with a single human V_H gene segments, a replacement of all or substantially all mouse D_H gene segments and J_H gene segments with human D_H and human J_H gene segments; wherein the mouse lacks a C_H1 and/or hinge region. In one embodiment, the mouse makes a single variable domain binding protein that is a dimer of immunoglobulin chains selected from: (a) human V_H – mouse C_H1 – mouse C_H2 – mouse C_H3; (b) human V_H –mouse hinge – mouse C_H2 – mouse C_H3; and, (c) human V_H – mouse C_H2 – mouse C_H3.

[00386] In one aspect, the nucleotide sequence that rescues fertility is placed upstream (or 5') of a human immunoglobulin heavy chain variable region sequence (*e.g.*, upstream of a human V_H1-2 or V_H1-69 gene segment) in a mouse that has a replacement of one or more mouse immunoglobulin heavy chain variable gene segments (mV_H's, mD_H's, and/or mJ_H's) with one or more human immunoglobulin heavy chain variable gene segments (hV_H's, hD_H's, and/or hJ_H's), and the mouse further comprises a replacement of one or more mouse immunoglobulin κ light chain variable gene segments (mVκ's and/or mJκ's) with one or more human immunoglobulin κ light chain variable gene segments (hVκ's and/or hJκ's).

[00387] In one aspect, the nucleotide sequence that rescues fertility is placed within a human immunoglobulin heavy chain variable region sequence (*e.g.*, between human V_H1-69 or human V_H1-2 and a human D_H1-1 gene segment) in a mouse that has a replacement of one or more mouse immunoglobulin heavy chain variable gene segments (mV_H's, mD_H's, and/or mJ_H's) with one or more human immunoglobulin heavy chain variable gene segments (hV_H's, hD_H's, and/or hJ_H's), and the mouse further comprises a replacement of one or more mouse immunoglobulin κ light chain

variable gene segments (mVκ's and/or mJκ's) with one or more human immunoglobulin κ light chain variable gene segments (hVκ's and/or hJκ's).

[00388] In one embodiment, the one or more mouse immunoglobulin heavy chain variable gene segments comprises about three megabases of the mouse immunoglobulin heavy chain locus. In one embodiment, the one or more mouse immunoglobulin heavy chain variable gene segments comprises at least 89 V_H gene segments, at least 13 D_H gene segments, at least four J_H gene segments or a combination thereof of the mouse immunoglobulin heavy chain locus. In one embodiment, the one or more human immunoglobulin heavy chain variable gene segments comprises a restricted number of (*e.g.*, one, two or three) V_H gene segments, at least 27 D_H gene segments, at least six J_H gene segments or a combination thereof of a human immunoglobulin heavy chain locus. In a specific embodiment, the restricted number of human V_H gene segments is one.

[00389] In one embodiment, the one or more mouse immunoglobulin κ light chain variable gene segments comprises about three megabases of the mouse immunoglobulin κ light chain locus. In one embodiment, the one or more mouse immunoglobulin κ light chain variable gene segments comprises at least 137 Vκ gene segments, at least five Jκ gene segments or a combination thereof of the mouse immunoglobulin κ light chain locus. In one embodiment, the one or more human immunoglobulin κ light chain variable gene segments comprises about one-half megabase of a human immunoglobulin κ light chain locus. In a specific embodiment, the one or more human immunoglobulin κ light chain variable gene segments comprises the proximal repeat (with respect to the immunoglobulin κ constant region) of a human immunoglobulin κ light chain locus. In one embodiment, the one or more human immunoglobulin κ light chain variable gene segments comprises at least 40Vκ gene segments, at least five Jκ gene segments or a combination thereof of a human immunoglobulin κ light chain locus.

[00390] In one embodiment, the nucleotide sequence is place between two human immunoglobulin gene segments. In a specific embodiment, the two human immunoglobulin gene segments are heavy chain gene segments. In one embodiment, the nucleotide sequence is placed between a human V_H1-69 gene segment and a human D_H1-1 gene segment. In one embodiment, the nucleotide sequence is placed between a human V_H12 gene segment and a human D_H1-1 gene segment. In one embodiment, the mouse so modified comprises a replacement of mouse immunoglobulin heavy chain variable gene segments with a single human V_H gene segments, 27 human D_H gene segments and six human J_H gene segments,

and a replacement of mouse immunoglobulin κ light chain variable gene segments with at least 40 human Vκ gene segments and five human Jκ gene segments.

[00391] In one aspect, a functional mouse ADAM6 locus (or ortholog or homolog or functional fragment thereof) is present in the midst of mouse gene segments that are present at the endogenous mouse heavy chain variable region locus, said locus incapable of rearranging to encode a functional heavy chain containing an endogenous heavy chain constant region. In one embodiment, the endogenous mouse heavy chain locus comprises at least one and up to 89 V_H gene segments, at least one and up to 13 D_H gene segments, at least one and up to four J_H gene segments and a combination thereof. In various embodiments, a functional mouse ADAM6 locus (or ortholog or homolog or functional fragment thereof) encodes one or more ADAM6 proteins that are functional in the mouse, wherein the one or more ADAM6 proteins comprise SEQ ID NO: 1, SEQ ID NO: 2 and/or a combination thereof.

[00392] In one aspect, a functional mouse ADAM6 locus (or ortholog or homolog or functional fragment thereof) is present in the midst of human gene segments that replace endogenous mouse gene segments. In one embodiment, at least 89 mouse V_H gene segments are removed and replaced with one, two or three human V_H gene segments, and the mouse ADAM6 locus is present immediately adjacent to the 3' end of the human V_H gene segments, or between two human V_H gene segments. In one embodiment, at least 89 mouse V_H gene segments are removed and replaced with a single human V_H gene segment, and the mouse ADAM6 locus is present immediately adjacent to the 3' end of the human V_H gene segment. In a specific embodiment, the mouse ADAM6 locus is present 3' of the V_H gene segment within about 20 kilo bases (kb) to about 40 kilo bases (kb) of the 3' terminus of the inserted human V_H gene segment. In a specific embodiment, the mouse ADAM6 locus is present 3' of the V_H gene segment within about 29 kb to about 31 kb of the 3' terminus of the inserted human V_H gene segment. In a specific embodiment, the mouse ADAM6 locus is present within about 30 kb of the 3' terminus of the inserted human V_H gene segment. In a specific embodiment, the mouse ADAM6 locus is present within about 30,184 bp of the 3' terminus of the inserted human V_H gene segment.

[00393] In a specific embodiment, the replacement includes human gene segments V_H1-69 and D_H1-1, and the mouse ADAM6 locus is present downstream of the V_H1-69 gene segment and upstream of the D_H1-1 gene segment. In a specific embodiment, the mouse ADAM6 locus is present between a human V_H1-69 gene

segment and a human D_H1-1 gene segment, wherein the 5' end of the mouse ADAM6 locus is about 258 bp from the 3' terminus of the human V_H1-69 gene segment and the 3' end of the ADAM6 locus is about 3,263 bp 5' of the human D_H1-1 gene segment. In a specific embodiment, the mouse ADAM6 locus comprises SEQ ID NO:3 or a fragment thereof that confers ADAM6 function within cells of the mouse. In a specific embodiment, the mouse ADAM6 locus comprises SEQ ID NO: 73 or a fragment thereof that confers ADAM6 function within cells of the mouse. In a specific embodiment, the mouse ADAM6 locus comprises SEQ ID NO: 77 or a fragment thereof that confers ADAM6 function within cells of the mouse. In a specific embodiment, the arrangement of human gene segments is then the following (from upstream to downstream with respect to direction of transcription of the human gene segments): human V_H1-69–mouse ADAM6 locus–human D_H1-1. In one embodiment, the orientation of one or more of mouse ADAM6a and mouse ADAM6b of the mouse ADAM6 locus is opposite with respect to direction of transcription as compared with the orientation of the human gene segments. Alternatively, the mouse ADAM6 locus is present 5' to, or upstream of, the single human V_H gene segment.

[00394] In a specific embodiment, the replacement includes human gene segments V_H1-2 and D_H1-1, and the mouse ADAM6 locus is present upstream of the V_H1-2 gene segment and upstream of the D_H1-1 gene segment. In a specific embodiment, the mouse ADAM6 locus is present upstream, or 5', of a human V_H1-2 gene segment and a human D_H1-1 gene segment, wherein the 5' end of the mouse ADAM6 locus is about 32,833 bp from the 5' terminus of the human V_H1-2 gene segment and the 3' end of the ADAM6 locus is about 18,078 bp from the 5' terminus of the human V_H1-2 gene segment. In a specific embodiment, the mouse ADAM6 locus comprises SEQ ID NO:3 or a fragment thereof that confers ADAM6 function within cells of the mouse. In a specific embodiment, the mouse ADAM6 locus comprises SEQ ID NO: 73 or a fragment thereof that confers ADAM6 function within cells of the mouse. In a specific embodiment, the mouse ADAM6 locus comprises SEQ ID NO: 77 or a fragment thereof that confers ADAM6 function within cells of the mouse. In a specific embodiment, the arrangement of human gene segments is then the following (from upstream to downstream with respect to direction of transcription of the human gene segments): mouse ADAM6 locus– human V_H1-2–human D_H1-1. In one embodiment, the orientation of one or more of mouse ADAM6a and mouse ADAM6b of the mouse ADAM6 locus is opposite with respect to direction of transcription as compared with the orientation of the human gene segments.

Alternatively, the mouse ADAM6 locus is present 3' to, or downstream of, the single human V_H gene segment.

[00395] Similarly, a mouse modified with one or more human V_L gene segments (*e.g.*, Vκ or Vλ segments) replacing all or substantially all endogenous mouse V_H gene segments can be modified so as to either maintain the endogenous mouse ADAM6 locus, as described above, *e.g.*, by employing a targeting vector having a downstream homology arm that includes a mouse ADAM6 locus or functional fragment thereof, or to replace a damaged mouse ADAM6 locus with an ectopic sequence positioned between two human V_L gene segments or between the human V_L gene segments and a D_H gene segment (whether human or mouse, *e.g.*, Vλ + m/hD_H), or a J gene segment (whether human or mouse, e.g., Vκ + J_H). In one embodiment, the replacement includes two or more human V_L gene segments, and the mouse ADAM6 locus or functional fragment thereof is present between the two 3'-most V_L gene segments. In a specific embodiment, the arrangement of human V_L gene segments is then the following (from upstream to downstream with respect to direction of transcription of the human gene segments): human V_L3'-1 – mouse ADAM6 locus – human V_L3'. In one embodiment, the orientation of one or more of mouse ADAM6a and mouse ADAM6b of the mouse ADAM6 locus is opposite with respect to direction of transcription as compared with the orientation of the human VL gene segments. Alternatively, the mouse ADAM6 locus is present in the intergenic region between the 3'-most human V_L gene segment and the 5'-most D_H gene segment. This can be the case whether the 5'-most D_H segment is mouse or human.

[00396] In one aspect, a mouse is provided with a replacement of one or more endogenous mouse V_H gene segments, and that comprises at least one endogenous mouse D_H gene segment. In such a mouse, the modification of the endogenous mouse V_H gene segments can comprise a modification of one or more of the 3'-most V_H gene segments, but not the 5'-most D_H gene segment, where care is taken so that the modification of the one or more 3'-most V_H gene segments does not disrupt or render the endogenous mouse ADAM6 locus nonfunctional. For example, in one embodiment the mouse comprises a replacement of all or substantially all endogenous mouse V_H gene segments with a single human V_H gene segment, and the mouse comprises one or more endogenous D_H gene segments and a functional endogenous mouse ADAM6 locus.

[00397] In another embodiment, the mouse comprises the modification of endogenous mouse 3'-most V_H gene segments, and a modification of one or more endogenous mouse D_H gene segments, and the modification is carried out so as to

maintain the integrity of the endogenous mouse ADAM6 locus to the extent that the endogenous ADAM6 locus remains functional. In one example, such a modification is done in two steps: (1) replacing the 3'-most endogenous mouse V_H gene segments with a single human V_H gene segments employing a targeting vector with an upstream homology arm and a downstream homology arm wherein the downstream homology arm includes all or a portion of a functional mouse ADAM6 locus; (2) then replacing and endogenous mouse D_H gene segment with a targeting vector having an upstream homology arm that includes a all or a functional portion of a mouse ADAM6 locus.

[00398] In various aspects, employing mice that contain an ectopic sequence that encodes a mouse ADAM6 protein or an ortholog or homolog or functional homolog thereof are useful where modifications disrupt the function of endogenous mouse ADAM6. The probability of disrupting endogenous mouse ADAM6 function is high when making modifications to mouse immunoglobulin loci, in particular when modifying mouse immunoglobulin heavy chain variable regions and surrounding sequences. Therefore, such mice provide particular benefit when making mice with immunoglobulin heavy chain loci that are deleted in whole or in part, are humanized in whole or in part, or are replaced (*e.g.*, with $V\kappa$ or $V\lambda$ sequences) in whole or in part. Methods for making the genetic modifications described for the mice described below are known to those skilled in the art.

[00399] Mice containing an ectopic sequence encoding a mouse ADAM6 protein, or a substantially identical or similar protein that confers the fertility benefits of a mouse ADAM6 protein, are particularly useful in conjunction with modifications to a mouse immunoglobulin heavy chain variable gene locus that disrupt or delete the endogenous mouse ADAM6 sequence. Although primarily described in connection with mice that express antibodies with human variable regions and mouse constant regions, such mice are useful in connection with any genetic modifications that disrupt endogenous mouse ADAM6 genes. Persons of skill will recognize that this encompasses a wide variety of genetically modified mice that contain modifications of mouse immunoglobulin heavy chain variable gene loci. These include, for example, mice with a deletion or a replacement of all or a portion of mouse immunoglobulin heavy chain gene segments, regardless of other modifications. Non-limiting examples are described below.

[00400] In some aspects, genetically modified mice are provided that comprise an ectopic mouse, rodent, or other ADAM6 gene (or ortholog or homolog or fragment) functional in a mouse, and one or more human immunoglobulin variable and/or constant region gene segments. In various embodiments, other ADAM6 gene

orthologs or homologs or fragments functional in a mouse may include sequences from bovine, canine, primate, rabbit or other non-human sequences.

[00401] In one aspect, a mouse is provided that comprises an ectopic ADAM6 sequence that encodes a functional ADAM6 protein, a replacement of all or substantially all mouse V_H gene segments with a single human V_H gene segment; a replacement of all or substantially all mouse D_H gene segments with one or more human D_H gene segments; and a replacement of all or substantially all mouse J_H gene segments with one or more human J_H gene segments.

[00402] In one embodiment, the mouse further comprises a replacement of a mouse C_H1 nucleotide sequence with a human C_H1 nucleotide sequence. In one embodiment, the mouse further comprises a replacement of a mouse hinge nucleotide sequence with a human hinge nucleotide sequence. In one embodiment, the mouse further comprises a replacement of an immunoglobulin light chain variable locus (V_L and J_L) with a human immunoglobulin light chain variable locus. In one embodiment, the mouse further comprises a replacement of a mouse immunoglobulin light chain constant region nucleotide sequence with a human immunoglobulin light chain constant region nucleotide sequence. In a specific embodiment, the V_L, J_L, and C_L are immunoglobulin κ light chain sequences. In a specific embodiment, the mouse comprises a mouse C_H2 and a mouse C_H3 immunoglobulin constant region sequence fused with a human hinge and a human C_H1 sequence, such that the mouse immunoglobulin loci rearrange to form a gene that encodes a binding protein comprising (a) a heavy chain that has a human variable region, a human C_H1 region, a human hinge region, and a mouse C_H2 and a mouse C_H3 region; and (b) a gene that encodes an immunoglobulin light chain that comprises a human variable domain and a human constant region.

[00403] In one aspect, a mouse is provided that comprises an ectopic ADAM6 sequence that encodes a functional ADAM6 protein, a replacement of all or substantially all mouse V_H gene segments with one or more human V_L gene segments, and optionally a replacement of all or substantially all D_H gene segments and/or J_H gene segments with one or more human D_H gene segments and/or human J_H gene segments, or optionally a replacement of all or substantially all D_H gene segments and J_H gene segments with one or more human J_L gene segments.

[00404] In one embodiment, the mouse comprises a replacement of all or substantially all mouse V_H, D_H, and J_H gene segments with one or more V_L, one or more D_H, and one or more J gene segments (*e.g.*, Jκ or Jλ), wherein the gene segments are operably linked to an endogenous mouse hinge region, wherein the

mouse forms a rearranged immunoglobulin chain gene that contains, from 5' to 3' in the direction of transcription, human V_L – human or mouse D_H – human or mouse J – mouse hinge – mouse C_H2 – mouse C_H3. In one embodiment, the J region is a human Jκ region. In one embodiment, the J region is a human J_H region. In one embodiment, the J region is a human Jλ region. In one embodiment, the human V_L region is selected from a human Vλ region and a human Vκ region.

[00405] In specific embodiments, the mouse expresses a single variable domain antibody having a mouse or human constant region and a variable region derived from a human Vκ, a human D_H and a human Jκ; a human Vκ, a human D_H, and a human J_H; a human Vλ, a human D_H, and a human Jλ; a human Vλ, a human D_H, and a human J_H; a human Vκ, a human D_H, and a human Jλ; a human Vλ, a human D_H, and a human Jκ. In specific embodiment, recombination recognition sequences are modified so as to allow for productive rearrangements to occur between recited V, D, and J gene segments or between recited V and J gene segments.

[00406] In one aspect, a mouse is provided that comprises an ectopic ADAM6 sequence that encodes a functional ADAM6 protein (or ortholog or homolog or functional fragment thereof), a replacement of all or substantially all mouse V_H gene segments with one or more human V_L gene segments, a replacement of all or substantially all mouse D_H gene segment and J_H gene segments with human J_L gene segments; wherein the mouse lacks a C_H1 and/or hinge region.

[00407] In one embodiment, the mouse lacks a sequence encoding a C_H1 domain. In one embodiment, the mouse lacks a sequence encoding a hinge region. In one embodiment, the mouse lacks a sequence encoding a C_H1 domain and a hinge region.

[00408] In a specific embodiment, the mouse expresses a binding protein that comprises a human immunoglobulin light chain variable domain (λ or κ) fused to a mouse C_H2 domain that is attached to a mouse C_H3 domain.

[00409] In one aspect, a mouse is provided that comprises an ectopic ADAM6 sequence that encodes a functional ADAM6 protein (or ortholog or homolog or functional fragment thereof), a replacement of all or substantially all mouse V_H gene segments with one or more human V_L gene segments, a replacement of all or substantially all mouse D_H and J_H gene segments with human J_L gene segments.

[00410] In one embodiment, the mouse comprises a deletion of an immunoglobulin heavy chain constant region gene sequence encoding a C_H1 region, a hinge region, a C_H1 and a hinge region, or a C_H1 region and a hinge region and a C_H2 region.

[00411] In one embodiment, the mouse makes a single variable domain binding protein comprising a homodimer selected from the following: (a) human V_L – mouse C_H1 – mouse C_H2 – mouse C_H3; (b) human V_L – mouse hinge – mouse C_H2 – mouse C_H3; (c) human V_L – mouse C_H2 – mouse C_H3.

[00412] In one aspect, a mouse is provided with a disabled endogenous heavy chain immunoglobulin locus, comprising a disabled or deleted endogenous mouse ADAM6 locus, wherein the mouse comprises a nucleic acid sequence that expresses a human or mouse or human/mouse or other chimeric antibody. In one embodiment, the nucleic acid sequence is present on a transgene integrated that is randomly integrated into the mouse genome. In one embodiment, the nucleic acid sequence is on an episome (*e.g.*, a chromosome) not found in a wild-type mouse.

[00413] In one embodiment, the mouse further comprises a disabled endogenous immunoglobulin light chain locus. In a specific embodiment, the endogenous immunoglobulin light chain locus is selected from a kappa (κ) and a lambda (λ) light chain locus. In a specific embodiment, the mouse comprises a disabled endogenous κ light chain locus and a disabled λ light chain locus, wherein the mouse expresses an antibody that comprises a human immunoglobulin heavy chain variable domain and a human immunoglobulin light chain domain. In one embodiment, the human immunoglobulin light chain domain is selected from a human κ light chain domain and a human λ light chain domain.

[00414] In one aspect, a genetically modified animal is provided that expresses a chimeric antibody and expresses an ADAM6 protein or ortholog or homolog thereof that is functional in the genetically modified animal.

[00415] In one embodiment, the genetically modified animal is selected from a mouse and a rat. In one embodiment, the genetically modified animal is a mouse, and the ADAM6 protein or ortholog or homolog thereof is from a mouse strain that is a different strain than the genetically modified animal. In one embodiment, the genetically modified animal is a rodent of family Cricetidae (*e.g.*, a hamster, a New World rat or mouse, a vole), and the ADAM6 protein ortholog or homolog is from a rodent of family Muridae (*e.g.*, true mouse or rat, gerbil, spiny mouse, crested rat). In one embodiment, the genetically modified animal is a rodent of the family Muridae, and the ADAM6 protein ortholog or homolog is from a rodent of family Cricetidae.

[00416] In one embodiment, the chimeric antibody comprises a human variable domain and a constant region sequence of a rodent. In one embodiment, the rodent is selected from a rodent of the family Cricetidae and a rodent of family Muridae. In a specific embodiment, the rodent of the family Cricetidae and of the family Muridae is

a mouse. In a specific embodiment, the rodent of the family Cricetidae and of the family Muridae is a rat. In one embodiment, the chimeric antibody comprises a human variable domain and a constant domain from an animal selected from a mouse or rat; in a specific embodiment, the mouse or rat is selected from the family Cricetidae and the family Muridae. In one embodiment, the chimeric antibody comprises a human heavy chain variable domain, a human light chain variable domain and a constant region sequence derived from a rodent selected from mouse and rat, wherein the human heavy chain variable domain and the human light chain are cognate. In a specific embodiment, cognate includes that the human heavy chain and the human light chain variable domains are from a single B cell that expresses the human light chain variable domain and the human heavy chain variable domain together and present the variable domains together on the surface of an individual B cell.

[00417] In one embodiment, the chimeric antibody is expressed from an immunoglobulin locus. In one embodiment, the heavy chain variable domain of the chimeric antibody is expressed from a rearranged endogenous immunoglobulin heavy chain locus. In one embodiment, the light chain variable domain of the chimeric antibody is expressed from a rearranged endogenous immunoglobulin light chain locus. In one embodiment, the heavy chain variable domain of the chimeric antibody and/or the light chain variable domain of the chimeric antibody is expressed from a rearranged transgene (*e.g.*, a rearranged nucleic acid sequence derived from an unrearranged nucleic acid sequence integrated into the animal's genome at a locus other than an endogenous immunoglobulin locus). In one embodiment, the light chain variable domain of the chimeric antibody is expressed from a rearranged transgene (*e.g.*, a rearranged nucleic acid sequence derived from an unrearranged nucleic acid sequence integrated into the animal's genome at a locus other than an endogenous immunoglobulin locus).

[00418] In a specific embodiment, the transgene is expressed from a transcriptionally active locus, *e.g.*, a ROSA26 locus, *e.g.*, a murine (*e.g.*, mouse) ROSA26 locus.

[00419] In one aspect, a non-human animal is provided, comprising a humanized immunoglobulin heavy chain locus, wherein the humanized immunoglobulin heavy chain locus comprises a non-human ADAM6 sequence or ortholog or homolog thereof.

[00420] In one embodiment, the non-human ADAM6 ortholog or homolog is a sequence that is orthologous and/or homologous to a mouse ADAM6 sequence, wherein the ortholog or homolog is functional in the non-human animal.

[00421] In one embodiment, the non-human animal is a rodent selected from a mouse, a rat, and a hamster.

[00422] In one embodiment, the non-human animal is selected from a mouse, a rat, and a hamster and the ADAM6 ortholog or homolog is from a non-human animal selected from a mouse, a rat, and a hamster. In a specific embodiment, the non-human animal is a mouse and the ADAM6 ortholog or homolog is from an animal that is selected from a different mouse species, a rat, and a hamster. In specific embodiment, the non-human animal is a rat, and the ADAM6 ortholog or homolog is from a rodent that is selected from a different rat species, a mouse, and a hamster. In a specific embodiment, the non-human animal is a hamster, and the ADAM6 ortholog or homolog is form a rodent that is selected from a different hamster species, a mouse, and a rat.

[00423] In a specific embodiment, the non-human animal is from the suborder Myomorpha, and the ADAM6 sequence is from an animal selected from a rodent of superfamily Dipodoidea and a rodent of the superfamily Muroidea. In a specific embodiment, the rodent is a mouse of superfamily Muroidea, and the ADAM6 ortholog or homolog is from a mouse or a rat or a hamster of superfamily Muroidea.

[00424] In one embodiment, the humanized heavy chain locus comprises a single human V_H gene segment, one or more human D_H gene segments and one or more human J_H gene segments. In a specific embodiment, the human V_H gene segment, one or more human D_H gene segments and one or more human J_H gene segments are operably linked to one or more human, chimeric and/or rodent (*e.g.*, mouse or rat) constant region genes. In one embodiment, the constant region genes are mouse. In one embodiment, the constant region genes are rat. In one embodiment, the constant region genes are hamster. In one embodiment, the constant region genes comprise a sequence selected from a hinge, a C_H2, a C_H3, and a combination thereof. In specific embodiment, the constant region genes comprise a hinge, a C_H2, and a C_H3 sequence.

[00425] In one embodiment, the non-human ADAM6 sequence is contiguous with a human immunoglobulin heavy chain sequence. In one embodiment, the non-human ADAM6 sequence is positioned within a human immunoglobulin heavy chain sequence. In a specific embodiment, the human immunoglobulin heavy chain sequence comprises a V, D and/or J gene segment.

[00426] In one embodiment, the non-human ADAM6 sequence is juxtaposed with a V gene segment. In one embodiment, the non-human ADAM6 sequence is positioned between two V gene segments. In one embodiment, the non-human ADAM6 sequence is juxtaposed between a V and a D gene segment. In one

embodiment, the mouse ADAM6 sequence is positioned between a V and a J gene segment. In one embodiment, the mouse ADAM6 sequence is juxtaposed between a D and a J gene segment.

[00427] In one aspect, a genetically modified non-human animal is provided, comprising a B cell that expresses a human V_H domain cognate with a human V_L domain from an immunoglobulin locus, wherein the non-human animal expresses a non-immunoglobulin non-human protein from the immunoglobulin locus. In one embodiment, the non-immunoglobulin non-human protein is an ADAM protein. In a specific embodiment, the ADAM protein is an ADAM6 protein or homolog or ortholog or functional fragment thereof.

[00428] In one embodiment the non-human animal is a rodent (*e.g.*, mouse or rat). In one embodiment, the rodent is of family Muridae. In one embodiment, the rodent is of subfamily Murinae. In a specific embodiment, the rodent of subfamily Murinae is selected from a mouse and a rat.

[00429] In one embodiment, the non-immunoglobulin non-human protein is a rodent protein. In one embodiment, the rodent is of family Muridae. In one embodiment, the rodent is of subfamily Murinae. In a specific embodiment, the rodent is selected from a mouse, a rat, and a hamster.

[00430] In one embodiment, the human V_H and V_L domains are attached directly or through a linker to an immunoglobulin constant domain sequence. In a specific embodiment, the constant domain sequence comprises a sequence selected from a hinge, a C_H2 a C_H3, and a combination thereof. In a specific embodiment, the human V_L domain is selected from a $V\kappa$ or a $V\lambda$ domain.

[00431] In one aspect, a genetically modified non-human animal is provided, comprising in its germline a human immunoglobulin sequence, wherein the sperm of a male non-human animal is characterized by an in vivo migration defect. In one embodiment, the in vivo migration defect comprises an inability of the sperm of the male non-human animal to migrate from a uterus through an oviduct of a female non-human animal of the same species. In one embodiment, the non-human animal lacks a nucleotide sequence that encodes and ADAM6 protein or functional fragment thereof. In a specific embodiment, the ADAM6 protein or functional fragment thereof includes an ADAM6a and/or an ADAM6b protein or functional fragments thereof. In one embodiment, the non-human animal is a rodent. In a specific embodiment, the rodent is selected from a mouse, a rat, and a hamster.

[00432] In one aspect, a non-human animal is provided, comprising a human immunoglobulin sequence contiguous with a non-human sequence that encodes an ADAM6 protein or ortholog or homolog or functional fragment thereof. In one

embodiment, the non-human animal is a rodent. In a specific embodiment, the rodent is selected from a mouse, a rat, and a hamster.

[00433] In one embodiment, the human immunoglobulin sequence is an immunoglobulin heavy chain sequence. In one embodiment, the immunoglobulin sequence comprises a single V_H gene segments. In one embodiment, the human immunoglobulin sequence comprises one or more D_H gene segments. In one embodiment, the human immunoglobulin sequence comprises one or more J_H gene segments. In one embodiment, the human immunoglobulin sequence comprises a single V_H gene segments, one or more D_H gene segments and one or more J_H gene segments.

[00434] In one embodiment, the immunoglobulin sequence comprises a single V_H gene segment that is associated with polymorphism in natural human repertoires. In a specific embodiment, the single V_H gene segment is selected from human V_H1-2, V_H1-69, V_H2-26, V_H2-70, or V_H3-23. In another specific embodiment the single V_H gene segment is V_H1-2. In another specific embodiment, the single V_H gene segment is V_H1-69.

[00435] In one embodiment, the immunoglobulin sequence comprises a single V_H gene segment that is associated with multiple copy number in natural human repertoires. In a specific embodiment, the single V_H gene segment is selected from human V_H1-2, V_H1-69, V_H2-26, V_H2-70, or V_H3-23. In another specific embodiment the single V_H gene segment is V_H1-2. In another specific embodiment, the single V_H gene segment is V_H1-69.

[00436] In various embodiments, the V_H gene segment is selected from V_H6-1, V_H1-2, V_H1-3, V_H2-5, V_H3-7, V_H1-8, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H1-18, V_H3-20, V_H3-21, V_H3-23, V_H1-24, V_H2-26, V_H4-28, V_H3-30, V_H4-31, V_H3-33, V_H4-34, V_H3-35, V_H3-38, V_H4-39, V_H3-43, V_H1-45, V_H1-46, V_H3-48, V_H3-49, V_H5-51, V_H3-53, V_H1-58, V_H4-59, V_H4-61, V_H3-64, V_H3-66, V_H1-69, V_H2-70, V_H3-72, V_H3-73 and V_H3-74.

[00437] In various embodiments, the V_H gene segment is selected from Table 1 and is represented in natural human repertoires by five or more alleles. In a specific embodiment the V_H gene is selected from V_H1-2, V_H1-69, V_H2-5, V_H2-70, V_H3-15, V_H3-23, V_H3-30, V_H3-33, V_H3-49, V_H3-64, V_H4-4, V_H4-28, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51 and V_H7-4-1.

[00438] In one embodiment, the non-human animal is a mouse, and the mouse comprises a replacement of endogenous mouse V_H gene segments with a single human V_H gene segments, wherein the human V_H gene segment is operably linked to a mouse C_H region gene, such that the mouse rearranges the human V_H gene

segment and expresses a reverse chimeric immunoglobulin heavy chain that comprises a human V_H domain and a mouse C_H. In one embodiment, 90-100% of unrearranged mouse V_H gene segments are replaced with one unrearranged human V_H gene segment. In a specific embodiment, all or substantially all of the endogenous mouse V_H gene segments are replaced with one unrearranged human V_H gene segment. In one embodiment, the replacement is with an unrearranged human V_H1-69 gene segment. In one embodiment, the replacement is with an unrearranged human V_H1-2 gene segment. In one embodiment, the replacement is with an unrearranged human V_H2-26 gene segment. In one embodiment, the replacement is with an unrearranged human V_H2-70 gene segment. In one embodiment, the replacement is with an unrearranged human V_H3-23 gene segment.

[00439] In one embodiment, the mouse comprises a replacement of all mouse 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 at least one unrearranged human D_H segment is selected from 1-1, 1-7, 1-26, 2-8, 2-15, 3-3, 3-10, 3-16, 3-22, 5-5, 5-12, 6-6, 6-13, 7-27, and a combination thereof. In one embodiment, the at least one unrearranged human J_H segment is selected from 1, 2, 3, 4, 5, 6, and a combination thereof.

[00440] In various embodiments, the human immunoglobulin sequence is in operable linkage with a constant region in the germline of the non-human animal (*e.g.*, the rodent, *e.g.*, the mouse, rat, or hamster). In one embodiment, the constant region is a human, chimeric human/mouse or chimeric human/rat or chimeric human/hamster, a mouse, a rat, or a hamster constant region. In one embodiment, the constant region is a rodent (*e.g.*, mouse or rat or hamster) constant region. In a specific embodiment, the rodent is a mouse or rat. In various embodiments, the constant region comprises at least a C_H2 domain and a C_H3 domain.

[00441] In one embodiment, the human immunoglobulin heavy chain sequence is located at an immunoglobulin heavy chain locus in the germline of the non-human animal (*e.g.*, the rodent, *e.g.*, the mouse or rat or hamster). In one embodiment, the human immunoglobulin heavy chain sequence is located at a non-immunoglobulin heavy chain locus in the germline of the non-human animal, wherein the non-heavy chain locus is a transcriptionally active locus. In a specific embodiment, the non-heavy chain locus is a ROSA26 locus.

[00442] In various aspects, the non-human animal further comprises a human immunoglobulin light chain sequence (*e.g.*, one or more unrearranged light chain V and J sequences, or one or more rearranged VJ sequences) in the germline of the non-human animal. In a specific embodiment, the immunoglobulin light chain

sequence is an immunoglobulin κ light chain sequence. In one embodiment, the human immunoglobulin light chain sequence comprises one or more V_L gene segments. In one embodiment, the human immunoglobulin light chain sequence comprises one or more J_L gene segments. In one embodiment, the human immunoglobulin light chain sequence comprises one or more V_L gene segments and one or more J_L gene segments. In a specific embodiment, the human immunoglobulin light chain sequence comprises at least 16 Vκ gene segments and five Jκ gene segments. In a specific embodiment, the human immunoglobulin light chain sequence comprises at least 30 Vκ gene segments and five Jκ gene segments. In a specific embodiment, the human immunoglobulin light chain sequence comprises at least 40 Vκ gene segments and five Jκ gene segments. In various embodiments, the human immunoglobulin light chain sequence is in operable linkage with a constant region in the germline of the non-human animal (*e.g.*, rodent, *e.g.*, mouse or rat or hamster). In one embodiment, the constant region is a human, chimeric human/rodent, mouse, rat, or hamster constant region. In a specific embodiment, the constant region is a mouse or rat constant region. In a specific embodiment, the constant region is a mouse κ constant (mCκ) region or a rat κ constant (rCκ) region.

[00443] In one embodiment, the non-human animal is a mouse and the mouse comprises a replacement of all or substantially all Vκ and Jκ gene segments with at least six human Vκ gene segments and at least one Jκ gene segment. In one embodiment, all or substantially all Vκ and Jκ gene segments are replaced with at least 16 human Vκ gene segments (human Vκ) and at least one Jκ gene segment. In one embodiment, all or substantially all Vκ and Jκ gene segments are replaced with at least 30 human Vκ gene segments and at least one Jκ gene segment. In one embodiment, all or substantially all Vκ and Jκ gene segments are replaced with at least 40 human Vκ gene segments and at least one Jκ gene segment. In one embodiment, the at least one Jκ gene segment comprises two, three, four, or five human Jκ gene segments.

[00444] In one embodiment, the human Vκ gene segments comprise Vκ4-1, Vκ5-2, Vκ7-3, Vκ2-4, Vκ1-5, and Vκ1-6. In one embodiment, the Vκ gene segments comprise Vκ3-7, Vκ1-8, Vκ1-9, Vκ2-10, Vκ3-11, Vκ1-12, Vκ1-13, Vκ2-14, Vκ3-15 and Vκ1-16. In one embodiment, the human Vκ gene segments comprise Vκ1-17, Vκ2-18, Vκ2-19, Vκ3-20, Vκ6-21, Vκ1-22, Vκ1-23, Vκ2-24, Vκ3-25, Vκ2-26, Vκ1-27, Vκ2-28, Vκ2-29, and Vκ2-30. In one embodiment, the human Vκ gene segments

comprise Vκ3-31, Vκ1-32, Vκ1-33, Vκ3-34, Vκ1-35, Vκ2-36, Vκ1-37, Vκ2-38, Vκ1-39, and Vκ2-40.

[00445] In a specific embodiment, the Vκ gene segments comprise contiguous human immunoglobulin κ gene segments spanning the human immunoglobulin κ light chain locus from Vκ4-1 through Vκ2-40, and the Jκ gene segments comprise contiguous gene segments spanning the human immunoglobulin κ light chain locus from Jκ1 through Jκ5.

[00446] In one embodiment, the human immunoglobulin light chain sequence is located at an immunoglobulin light chain locus in the germline of the non-human animal. In a specific embodiment, the immunoglobulin light chain locus in the germline of the non-human animal is an immunoglobulin κ light chain locus. In one embodiment, the human immunoglobulin light chain sequence is located at a non-immunoglobulin light chain locus in the germline of the non-human animal that is transcriptionally active. In a specific embodiment, the non-immunoglobulin locus is a ROSA26 locus.

[00447] In one aspect, a method of making a human antibody is provided, wherein the human antibody comprises variable domains derived from one or more variable region nucleic acid sequences encoded in a cell of a non-human animal as described herein.

[00448] In one aspect, a method of making an anti-idiotype antibody is provided, wherein the anti-idiotype antibody comprises variable domains derived from one or more variable region nucleic acid sequences encoded in a cell of a non-human animal as described herein, the method comprising exposing a non-human animal as described herein to an antibody comprising human variable domains. In one embodiment, the anti-idiotype antibody is specific for or is capable of binding a human heavy chain variable domain. In one embodiment, the antibody is specific for or is capable of binding a human light chain variable domain.

[00449] In a specific embodiment, the anti-idiotype antibody is specific for or is capable of binding a human heavy chain variable domain, wherein the human heavy chain variable domain comprises a rearranged human V_H gene segment selected from V_H6-1, V_H1-2, V_H1-3, V_H2-5, V_H3-7, V_H1-8, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H1-18, V_H3-20, V_H3-21, V_H3-23, V_H1-24, V_H2-26, V_H4-28, V_H3-30, V_H4-31, V_H3-33, V_H4-34, V_H3-35, V_H3-38, V_H4-39, V_H3-43, V_H1-45, V_H1-46, V_H3-48, V_H3-49, V_H5-51, V_H3-53, V_H1-58, V_H4-59, V_H4-61, V_H3-64, V_H3-66, V_H1-69, V_H2-70, V_H3-72, V_H3-73 and V_H3-74.

[00450] In a specific embodiment, the anti-idiotype antibody is specific for or is capable of binding a human heavy chain variable domain, wherein the human heavy chain variable domain comprises a rearranged human V_H gene segment selected from V_H1-2, V_H1-69, V_H2-5, V_H2-70, V_H3-15, V_H3-23, V_H3-30, V_H3-33, V_H3-49, V_H3-64, V_H4-4, V_H4-28, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51 and V_H7-4-1.

[00451] In a specific embodiment, the anti-idiotype antibody is specific for or is capable of binding a human light chain variable domain, wherein the human light chain variable domain comprises a rearranged human Vκ gene segment selected from Vκ4-1, Vκ5-2, Vκ7-3, Vκ2-4, Vκ1-5, Vκ1-6, Vκ3-7, Vκ1-8, Vκ1-9, Vκ2-10, Vκ3-11, Vκ1-12, Vκ1-13, Vκ2-14, Vκ3-15, Vκ1-16, Vκ1-17, Vκ2-18, Vκ2-19, Vκ3-20, Vκ6-21, Vκ1-22, Vκ1-23, Vκ2-24, Vκ3-25, Vκ2-26, Vκ1-27, Vκ2-28, Vκ2-29, Vκ2-30, Vκ3-31, Vκ1-32, Vκ1-33, Vκ3-34, Vκ1-35, Vκ2-36, Vκ1-37, Vκ2-38, Vκ1-39, and Vκ2-40.

[00452] In a specific embodiment, the anti-idiotype antibody is specific for or is capable of binding a human light chain variable domain, wherein the human light chain variable domain comprises a rearranged human Vκ1-39 gene segment.

[00453] In a specific embodiment, the anti-idiotype antibody is specific for or is capable of binding a human light chain variable domain, wherein the human light chain variable domain comprises a rearranged human Vλ gene segment selected from Vλ3-1, Vλ4-3, Vλ2-8, Vλ3-9, Vλ3-10, Vλ2-11, Vλ3-12, Vλ2-14, Vλ3-16, Vλ2-18, Vλ3-19, Vλ3-21, Vλ3-22, Vλ2-23, Vλ3-25, Vλ3-27, Vλ3-32, Vλ2-33, Vλ2-34, Vλ1-36, Vλ1-40, Vλ7-43, Vλ1-44, Vλ5-45, Vλ7-46, Vλ1-47, Vλ5-48, Vλ9-49, Vλ1-50, Vλ1-51, Vλ5-52, Vλ10-54, Vλ11-55, Vλ6-57, Vλ4-60, Vλ8-61, and Vλ4-69.

[00454] In one embodiment, a method of making an anti-idiotype antibody is provided, wherein the anti-idiotype antibody comprises variable domains derived from one or more variable region nucleic acid sequences encoded in a cell of a non-human animal that comprises a restricted immunoglobulin heavy chain locus comprising a single human V_H gene segment, 27 D_H gene segments, and six J_H gene segments, and wherein the anti-idiotype antibody is specific for or is capable of binding a human heavy chain variable domain comprising a rearranged human V_H1-69 gene segment, the method comprising exposing the non-human animal to an antibody comprising the rearranged human V_H1-69 gene segment and isolating the anti-idiotype antibody from the non-human animal. In a specific embodiment, the single human V_H gene segment is selected from a human V_H1-2 and a human V_H1-69 gene segment.

[00455] In one embodiment, a method of making an anti-idiotype antibody is provided, wherein the anti-idiotype antibody comprises variable domains derived from one or more variable region nucleic acid sequences encoded in a cell of a non-human animal that comprises a restricted immunoglobulin heavy chain locus comprising a single human V_H gene segment, 27 D_H gene segments, and six J_H gene segments, and wherein the anti-idiotype antibody is specific for or is capable of binding a human light chain variable domain comprising a rearranged human V_K1-39 gene segment, the method comprising exposing the non-human animal to an antibody comprising the human V_K1-39 gene segment and isolating the antibody from the non-human animal. In a specific embodiment, the single human V_H gene segment is selected from a human V_H1-2 and a human V_H1-69 gene segment.

[00456] In one aspect, a pharmaceutical composition is provided, comprising a polypeptide that comprises antibody or antibody fragment that is derived from one or more variable region nucleic acid sequences isolated from a non-human animal as described herein. In one embodiment, the polypeptide is an antibody. In one embodiment, the polypeptide is a heavy chain only antibody. In one embodiment, the polypeptide is a single chain variable fragment (*e.g.*, an scFv).

[00457] In one aspect, use of a non-human animal as described herein to make an antibody is provided. In various embodiments, the antibody comprises one or more variable domains that are derived from one or more variable region nucleic acid sequences isolated from the non-human animal. In a specific embodiment, the variable region nucleic acid sequences comprise immunoglobulin heavy chain gene segments. In a specific embodiment, the variable region nucleic acid sequences comprise immunoglobulin light chain gene segments.

EXAMPLES

[00458] 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. Unless indicated otherwise, temperature is indicated in Celsius, and pressure is at or near atmospheric.

Example 1

Construction of a Restricted Humanized IgH Locus

[00459] A uniquely engineered human heavy chain locus containing a single human V_H gene segment located upstream of all the human D_H and J_H gene

segments may be constructed by homologous recombination using Bacterial Artificial Chromosome (BAC) DNA. Exemplary human V_H gene segments employed for construction of such an immunoglobulin heavy chain locus include polymorphic V_H gene segments and/or V_H gene segments associated with a variation in copy number, such as, for example V_H1-2, V_H1-69, V_H2-26, V_H2-70, and V_H3-23. VELOCIGENE® genetic engineering technology can be employed for the creation of a single V_H containing heavy chain locus using several targeting constructs (see, e.g., US Pat. No. 6,586,251 and Valenzuela, D.M. *et al.*, 2003, High-throughput engineering of the mouse genome coupled with high-resolution expression analysis. *Nature Biotechnology* 21(6): 652-659).

[00460] Exemplary Strategy For Construction of a Human V_H1-69 Restricted IgH Locus (FIG. 1). In the first step, a modified human BAC containing multiple distal (5') human V_H gene segments, including V_H1-69, an upstream selection cassette (*e.g.*, hygromycin) and a 5' mouse homology arm was targeted by homologous recombination with a second selection cassette (*e.g.*, spectinomycin), which also contained a modified recombination signal sequence (Step 1, FIG. 1). This modified recombination signal sequence (RSS) introduced two point mutations (T to A and G to A) in the 3' RSS region of the human V_H1-69 gene changing the RSS nonamer to the optimal consensus sequence. Thus, Step 1 resulted in a human genomic fragment containing the human V_H1-69 gene segment with a modified 3' RSS, a unique AsiSI restriction site about 180 bp downstream of the RSS and a spectinomycin cassette.

[00461] Step 2 included the use of a neomycin (Neo) cassette flanked by Frt sites to delete the selection cassette (hygromycin) and additional upstream (5') human V_H gene segments. This modification was targeted, by homologous recombination, 5' to the human V_H1-69 gene segment to leave intact about 8.2 kb of the promoter region of human V_H1-69 and the 5' mouse homology arm.

[00462] Step 3 included another selection cassette (spectinomycin) flanked by uniquely engineered restriction sites (*e.g.*, PI-SceI and AsiSI) targeted by homologous recombination to a human genomic fragment containing the first three functional human V_H gene segments and all the human D_H and J_H gene segments (FIG. 1). The human genomic fragment was previously targeted by homologous recombination with a neomycin cassette and contained 5' and 3' homology arms containing the mouse genomic sequence 5' and 3' of the endogenous heavy chain locus including the 3' intronic enhancer and the IgM gene. This modification deleted the 5' mouse genomic sequence and human V_H gene segments, leaving about 3.3 kb of the V_H-D_H intergenic region upstream of the human D_H1-1 gene segment, all of the

human D_H and J_H segments, and the 3' mouse genomic fragment containing the 3' intronic enhancer and the IgM gene (FIG. 1).

[00463] Step 4 was accomplished by using the unique restriction sites (described above) to cut followed by ligation of the two modified BACs from Step 2 and Step 3, which yielded the final targeting construct. The final targeting construct for the creation of a modified heavy chain locus containing a human V_H1-69 gene segment, all the human D_H, and all the human J_H gene segments in ES cells contained, from 5' to 3', a 5' homology arm containing about 20 kb of mouse genomic sequence upstream of the endogenous heavy chain locus, a 5' Frt site, a neomycin cassette, a 3' Frt site, about 8.2 kb of the human V_H1-69 promoter, the human V_H1-69 gene segment with a modified 3' RSS, 27 human D_H gene segments, six human J_H segments, and a 3' homology arm containing about 8 kb of mouse genomic sequence downstream of the mouse J_H gene segments including the 3' intronic enhancer and IgM gene (FIG. 1).

[00464] Exemplary Strategy For Construction of a Human V_H1-2 Restricted IgH Locus (FIG. 2). In a similar fashion, other polymorphic V_H gene segments in the context of mouse heavy chain constant regions are employed to construct a series of mice having a restricted number immunoglobulin heavy chain V segments (*e.g.*, 1, 2, 3, 4, or 5), wherein the V segments are polymorphic variants of a V gene family member. Exemplary polymorphic V_H gene segments are derived from human V_H gene segments including, *e.g.*, V_H1-2, V_H2-26, V_H2-70 and V_H3-23. Such human V_H gene segments are obtained, *e.g.*, by de novo synthesis (*e.g.*, Blue Heron Biotechnology, Bothell, WA) using sequences available on published databases. Thus, DNA fragments encoding each V_H gene are, in some embodiments, generated independently for incorporation into targeting vectors, as described herein. In this way, multiple modified immunoglobulin heavy chain loci comprising a restricted number of V_H gene segments are engineered in the context of mouse heavy chain constant regions. An exemplary targeting strategy for creating a restricted humanized heavy chain locus containing a human V_H1-2 gene segment, 27 human D_H gene segments, and six human J_H gene segments is shown in FIG. 2.

[00465] Briefly, a modified human BAC clone containing three human V_H gene segments (V_H6-1, V_H1-2, V_H1-3), 27 human D_H gene segments, and six human J_H gene segments (see USSN 13/404,075; filed 24 February 2012) is used to create a restricted humanized heavy chain locus containing a human V_H1-2 gene segment. This modified BAC clone functionally links the aforementioned human heavy chain gene segments with the mouse intronic enhancer and the IgM constant region. The restricted human V_H1-2 based heavy chain locus is achieved by two homologous

recombinations using the modified human BAC clone described above. In the first homologous recombination, 205 bp of the human V_H6-1 gene segment (from about 10 bp upstream (5') of the V_H6-1 start codon in exon 1 to about 63 bp downstream (3') of the beginning of exon 2) in the modified human BAC clone is deleted by bacterial homologous recombination using a spectinomycin (aadA) cassette flanked by unique PI-SceI restriction sites (FIG. 2, BHR 1). This allows for subsequent removal of the aadA cassette without disrupting other human gene segments within the restricted heavy chain locus. In the second homologous recombination, the 5' end of the modified human BAC clone including the entire human V_H1-3 gene segment and about 60 bp downstream (3') of the gene segment is deleted by homologous recombination using a hygromycin cassette containing flanking 5' AsiSI and 3' AscI restriction sites (FIG. 2, BHR 2). As described above, the spectinomycin cassette is optionally removed after confirmation of the final targeting vector including deletion of the two human V_H gene segments flanking the human V_H1-2 gene segment (FIG. 2, bottom). An exemplary human V_H1-2 targeting vector is set forth in SEQ ID NO: 75.

[00466] Employing polymorphic V_H gene segments in a restricted immunoglobulin heavy chain locus represents a novel approach for generating antibodies, populations of antibodies, and populations of B cells that express antibodies having heavy chains with diverse CDRs derived from a single human V_H gene segment. Exploiting the somatic hypermutation machinery of the host animal along with combinatorial association with rearranged human immunoglobulin light chain variable domains results in the engineering of unique heavy chains and unique V_H/V_L pairs that expand the immune repertoire of genetically modified animals and enhance their usefulness as a next generation platform for making human therapeutics, especially useful as a platform for making neutralizing antibodies specific for human pathogens.

[00467] Based on the final desired locus structure, one of the other human V_H gene segments may be substituted in a similar fashion using human BAC clones containing the desired human V_H gene segment. Thus, using the strategy outlined above for incorporation of additional and/or other polymorphic V_H gene segments into the mouse immunoglobulin heavy chain locus allows for the generation of novel antibody repertoires for use in neutralizing human pathogens that might otherwise effectively evade the host immune system.

[00468] Targeted ES cells described above are used as donor ES cells and introduced into an 8-cell stage mouse embryo by the VELOCIMOUSE® method (*supra*). Mice bearing a humanized heavy chain locus containing a single human V_H gene segment, all the human D_H and J_H gene segments operably linked to the mouse

immunoglobulin constant region genes are identified by genotyping using a modification of allele assay (Valenzuela *et al., supra*) that detected the presence of the neomycin cassette, the human V_H gene segment and a region within the human D_H and J_H gene segments as well as endogenous heavy chain sequences. Table 3 sets forth the primers and probes that are used to confirm mice harboring a restricted heavy chain locus containing a single human V_H1-69 gene segment, 27 human D_H gene segments and six human J_H gene segments.

[00469] Mice bearing an engineered heavy chain locus that contains a single human V_H gene segment can be bred to a FLPe deletor mouse strain (see, e.g., Rodriguez, C.I. *et al.* (2000) High-efficiency deleter mice show that FLPe is an alternative to Cre-*lox*P. *Nature Genetics* 25: 139-140) in order to remove any Frt'ed neomycin cassette introduced by the targeting vector that is not removed, *e.g.*, at the ES cell stage or in the embryo. Optionally, the neomycin cassette is retained in the mice.

[00470] Pups are genotyped and a pup heterozygous for a humanized heavy chain locus containing a single human V_H gene segment, all the human D_H and J_H segments operably linked to the endogenous mouse immunoglobulin constant genes is selected for characterizing the immunoglobulin heavy chain repertoire.

Table 3

Name (Region Detected)	Sequence (5'-3')	SEQ ID NO:
hyg (hygromycin cassette)	Forward: TGCGGCCGAT CTTAGCC	7
	Reverse: TTGACCGATT CCTTGCGG	8
	Probe: ACGAGCGGGT TCGGCCCATT C	9
neo (neomycin cassette)	Forward: GGTGGAGAGG CTATTCGGC	10
	Reverse: GAACACGGCG GCATCAG	11
	Probe: TGGGCACAAC AGACAATCGG CTG	12
hIgH9T (human $D_H - J_H$ genomic sequence)	Forward: TCCTCCAACG ACAGGTCCC	13
	Reverse: GATGAACTGA CGGGCACAGG	14
	Probe: TCCCTGGAAC TCTGCCCCGA CACA	15
77h3 (human V_H1-69 gene segment)	Forward: CTCTGTGGAA AATGGTATGG AGATT	16
	Reverse: GGTAAGCATA GAAGGTGGGT ATCTTT	17
	Probe: ATAGAACTGT CATTTGGTCC AGCAATCCCA	18

mIgHA7 (mouse $D_H - J_H$ genomic sequence)	Forward: TGGTCACCTC CAGGAGCCTC Reverse: GCTGCAGGGT GTATCAGGTG C Probe: AGTCTCTGCT TCCCCCTTGT GGCTATGAGC	19 20 21
88710T (mouse 3' V_H genomic sequence)	Forward: GATGGGAAGA GACTGGTAAC ATTTGTAC Reverse: TTCCTCTATT TCACTCTTTG AGGCTC Probe: CCTCCACTGT GTTAATGGCT GCCACAA	22 23 24
mIgHd10 (mouse 5' V_H genomic sequence)	Forward: GGTGTGCGAT GTACCCTCTG AAC Reverse: TGTGGCAGTT TAATCCAGCT TTATC Probe: CTAAAAATGC TACACCTGGG GCAAAACACC TG	25 26 27
mIgHp2 (mouse J_H genomic sequence)	Forward: GCCATGCAAG GCCAAGC Reverse: AGTTCTTGAG CCTTAGGGTG CTAG Probe: CCAGGAAAAT GCTGCCAGAG CCTG	28 29 30

Example 2

Reengineering of ADAM Genes into a Restricted Humanized IgH Locus

[00471] Mice with humanized immunoglobulin heavy chain loci in which the endogenous variable region gene segments (VDJ) have been replaced and/or deleted lack expression of endogenous ADAM6 genes. In particular, male mice comprising such humanized immunoglobulin heavy chain loci demonstrate a reduction in fertility. Thus, the ability to express ADAM6 was reengineered into mice with humanized, yet restricted, heavy chain loci to perpetuate the modified mouse strains using normal breeding methods.

[00472] Reengineering of ADAM6 Genes into a Human V_H1-69 Restricted IgH Locus (FIG. 3). A restricted immunoglobulin heavy chain locus containing a single human V_H1-69 gene segment located upstream of all the human D_H and J_H gene segments was reengineered to contain a genomic fragment encoding mouse ADAM6a and ADAM6b (SEQ ID NO: 77) by homologous recombination using BAC DNA. This was accomplished by VELOCIGENE® genetic engineering technology (*supra*) in a series of six steps that included modification of BAC DNA containing mouse and human sequences that yielded a final targeting vector containing a restricted humanized heavy chain locus contiguous with mouse heavy chain constant regions and mouse ADAM6 genes.

[00473] First, a mouse genomic fragment that encoded mouse ADAM6a and ADAM6b was prepared for insertion into a humanized heavy chain locus containing a single V_H gene segments by a series of three bacterial homologous recombinations

involving different selection cassettes to uniquely position restriction sites around the mouse ADAM6 genes (FIG. 3, Steps 1 – 3). In the first step, mouse BAC DNA containing a portion of the mouse immunoglobulin heavy chain locus was targeted with a neomycin cassette flanked by recombination sites, which was engineered to contain unique AsiSI restriction sites. In the second step, the modified mouse fragment containing mouse ADAM6 genes and the neomycin cassette was then targeted to delete the mouse D_H and J_H gene segments and replace them with a spectinomycin cassette that contained a unique AscI restriction site positioned 5' of the selection gene. In the third step, the double modified mouse fragment containing a neomycin cassette positioned between the mouse ADAM6 genes and a spectinomycin cassette was targeted to swap out the neomycin cassette for a hygromycin cassette. This was carried out so that the modified mouse genomic fragment containing the ADAM6 genes could be inserted by ligation of compatible genomic fragments into a humanized heavy chain locus containing the single V_H gene segment.

[00474] In step four, a humanized heavy chain locus containing a human V_H1-69 gene segment, 27 human D_H gene segments, and six human J_H gene segments was separately targeted by bacterial homologous recombination with a spectinomycin cassette containing unique I-CeuI and AscI restriction sites at 5' and 3' locations in the cassette, respectively (FIG. 3, top left). Following this step, the modified genomic fragment containing a restricted humanized heavy chain locus, neomycin and spectinomycin cassettes and the modified mouse fragment containing the ADAM6 genes, hygromycin and spectinomycin cassettes were separately digested with I-CeuI and AscI restriction enzymes to create modified genomic fragments for ligation (FIG. 3, middle). In step five, the appropriate digested genomic fragments were purified and ligated together to yield a reengineered humanized heavy chain locus containing a single human V_H gene segment, 27 human D_H gene segments, six human J_H gene segments and an integrated mouse genomic fragment encoding ADAM6a and ADAM6b with neomycin and hygromycin resistance. In the final step (Step 6), the hygromycin cassette was deleted by AsiSI digestion followed by relegation of the compatible ends.

[00475] This step produced the final targeting vector for reinsertion of mouse ADAM6a and ADAM6b sequences into a restricted humanized heavy chain locus, which contained, from 5' to 3', a 5' homology arm containing about 20 kb of mouse genomic sequence upstream of the endogenous heavy chain locus, a 5' Frt site, a neomycin cassette, a 3' Frt site, about 8.2 kb of the human V_H1-69 promoter, the human V_H1-69 gene segment with a modified 3' RSS, a mouse genomic fragment

containing about 17711 bp of mouse genomic sequence including mouse ADAM6a and ADAM6b genes (SEQ ID NO: 77), a human genomic fragment containing 27 human D_H and six human J_H gene segments, and a 3' homology arm containing about 8 kb of mouse genomic sequence downstream of the endogenous heavy chain locus including the intronic enhancer and the IgM constant region gene (Human V_H1-69/A6 Targeting Vector, SEQ ID NO: 74; FIG. 3, bottom).

[00476] Reengineering of ADAM6 Genes Into a Human V_H1-2 Restricted IgH Locus (FIG. 4).

[00477] A restricted immunoglobulin heavy chain locus containing a single human V_H1-2 gene segment located upstream of all the human D_H and J_H gene segments is reengineered to contain a genomic fragment encoding mouse ADAM6a and ADAM6b (SEQ ID NO: 73) by homologous recombination using BAC DNA. This was accomplished by VELOCIGENE® genetic engineering technology (*supra*) in a series of steps that included modification of BAC DNA containing mouse and human sequences that yielded a final targeting vector containing a restricted humanized heavy chain locus contiguous with mouse heavy chain constant regions and mouse ADAM6 genes.

[00478] A modified human BAC clone containing a single human V_H1-2 gene segment flanked by 5' hygromycin and 3' spectinomycin cassettes, 27 human D_H gene segments, six human J_H gene segments, a mouse heavy chain intronic enhancer, and a mouse IgM constant region (described above in Example 1) was modified to contain a genomic fragment encoding mouse ADAM6 genes. This is accomplished by a modified isothermic DNA assembly method referred to herein as oligo-mediated isothermal assembly, which is based on the method described in Gibson *et al.* (2009, Enzymatic assembly of DNA molecules up to several hundred kilobases, *Nature Methods* 6(5):343-345). This modified method does not require sequence identity between the ligated fragments. Instead, sequence identity is imparted by an oligo that serves to join the two fragments. Further, the oligo serves as a template that adds sequence identity to the end of one of the fragments. The extended fragment enables hybridization with the second fragment. Specifically, oligo-mediated isothermal assembly was employed to replace the hygromycin cassette with a NotI-AscI fragment containing a 20 kb distal mouse IgH homology arm, the mouse ADAM6a gene, a neomycin cassette flanked by Frt sites, and the mouse ADAM6b gene.

[00479] Briefly, the modified human BAC clone containing a restricted human V_H1-2 heavy chain locus (FIG. 4, top left) is digested with AsiSI and AscI to remove the hygromycin cassette, and a modified mouse BAC containing the mouse ADAM6

genes (FIG. 4, top right) is digested with NotI and AscI to remove the fragment containing the 5' mouse arm and release the mouse ADAM6 genes flanking the neomycin cassette. The two digested BAC fragments are subsequently mixed together with 5' and 3' joiner oligonucleotides and incubated for 1 hour at 50°C in an assembly reaction mixture (T5 exonuclease, Phusion DNA polymerase, Taq DNA ligase, 10mM DTT, 5% PEG8000 (w/v), 1mM NAD, 0.2mM dNTPs, 10mM $MgCl_2$, and 100mM Tris-HCl). The 5' joiner oligo contains a 38 bp overlap with sequence 5' of the AsiSI site of the modified human BAC clone containing human V_H1-2, and a 30 bp overlap with the NotI site and adjacent 3' sequence of the modified mouse BAC clone containing ADAM6 genes. The 3' joiner oligo contains a 26 bp overlap with sequence 5' of the AscI site of modified mouse BAC clone containing ADAM6 genes, an AscI site, and a 35 bp overlap with sequence 3' of the AscI site of the modified human BAC clone containing human V_H1-2. The assembly reaction is transformed into *E. coli* and the correct product is selected with kanamycin and spectinomycin selection. To create the final targeting vector, the spectinomycin cassette is removed by PI-SceI digestion followed by religation.

[00480] The final targeting vector contains, from 5' to 3', a 20 kb distal mouse IgH homology arm, a mouse ADAM6a gene, a 5' Frt site, a neomycin cassette, a 3' Frt site, a mouse ADAM6b gene, a ~18 kb human genomic fragment, a human V_H1-2 gene segment, a ~46.6 kb human genomic fragment, an inactivated human V_H6-1 gene segment, 27 human D_H gene segments, six human J_H gene segments, and an 8 kb 3' mouse homology arm containing a mouse IgH intronic enhancer and IgM constant region (SEQ ID NO: 76)

[00481] Each of the final targeting vectors (described above) were used to electroporate mouse ES cells that contained a deleted endogenous heavy chain locus to created modified ES cells comprising a mouse genomic sequence ectopically placed that comprises mouse ADAM6a and ADAM6b sequences within a restricted humanized heavy chain locus. Positive ES cells containing the ectopic mouse genomic fragment within the humanized heavy chain locus were identified by a quantitative PCR assay using TAQMAN™ probes (Lie and Petropoulos, 1998, Advances in quantitative PCR technology: 5'nuclease assays, *Curr Opin Biotechnol* 9(1):43-48).

[00482] Targeted ES cells described above were used as donor ES cells and introduced into an 8-cell stage mouse embryo by the VELOCIMOUSE® mouse engineering method (see, *e.g.*, US Pat. Nos. 7,6598,442, 7,576,259, 7,294,754). Mice bearing a humanized heavy chain locus containing a restricted number of human gene segments and an ectopic mouse genomic sequence comprising mouse

ADAM6a and ADAM6b sequences were identified by genotyping using a modification of allele assay (Valenzuela *et al.*, 2003) that detected the presence of the mouse ADAM6a and ADAM6b genes within the restricted humanized heavy chain locus as well as human heavy chain sequences.

[00483] Pups are genotyped and a pup heterozygous for a restricted humanized heavy chain locus containing an ectopic mouse genomic fragment that comprises mouse ADAM6a and ADAM6b sequences is selected for characterizing mouse ADAM6 gene expression and fertility.

We claim:

1. A mouse having in its germline

(a) a human genomic sequence comprising a single human V_H gene segment, one or more D_H gene segments, and one or more J_H gene segments; and

(b) a sequence that encodes an ADAM6 protein that is functional in a male mouse, wherein the sequence that encodes the ADAM6 is located at a position different than an ADAM6 locus of a wild-type mouse.

2. The mouse of claim 1, wherein the single human V_H gene segment is a polymorphic V_H gene segment.

3. The mouse of claim 1, wherein the sequence that encodes the ADAM6 is a mouse sequence.

4. The mouse of claim 1, wherein the mouse genomic sequence is juxtaposed or contiguous with the human genomic sequence.

5. The mouse of claim 1, wherein the human genomic sequence and the mouse genomic sequence are present at an endogenous immunoglobulin heavy chain locus.

6. The mouse of claim 1, wherein the human genomic sequence is present at an endogenous immunoglobulin heavy chain locus and the mouse genomic sequence is present at a position in the mouse genome other than an endogenous immunoglobulin heavy chain locus.

7. The mouse of claim 1, wherein the mouse comprises a deletion of all or substantially all endogenous V_H gene segments.

8. The mouse of claim 1, wherein the single human V_H gene segment is selected from V_H1-2, V_H1-69, V_H2-26, V_H2-70, V_H3-23, or a polymorphic variant thereof.

9. The mouse of claim 8, wherein the human V_H gene segment is V_H1-69.

10. The mouse of claim 8, wherein the human V_H gene segment is V_H1-2.

11. The mouse of claim 1, wherein the single V_H gene segment is operably linked to a human, mouse, or chimeric human/mouse immunoglobulin constant region gene.

12. The mouse of claim 11, wherein the immunoglobulin constant region gene is a mouse constant region gene.

13. The mouse of claim 11, wherein the immunoglobulin constant gene comprises a human sequence selected from a human C_H1, a human hinge, a human C_H2, a human C_H3, and a combination thereof.

14. The mouse of claim 12, wherein the mouse constant gene is at an endogenous immunoglobulin heavy chain locus.

15. The mouse of claim 1, wherein the human genomic sequence comprises a human V_H1-69 gene segment, 27 human D_H gene segments, and six human J_H gene segments.

16. The mouse of claim 1, wherein the human genomic sequence comprises a human V_H1-2 gene segment, 27 human D_H gene segments, and six human J_H gene segments.

17. The mouse of claim 1, 15, or 16, further comprising one or more human $V\kappa$ gene segments and one or more $J\kappa$ gene segments.

18. The mouse of claim 17, wherein the one or more human $V\kappa$ gene segments and one or more $J\kappa$ gene segments are present at an endogenous immunoglobulin light chain locus.

19. A mouse comprising at least one human V_H gene segment operably linked to at least one human D_H gene segment and at least one human J_H gene segment, wherein the mouse comprises an ectopic mouse ADAM6 sequence.

20. The mouse of claim 19, wherein said nucleic acid sequence is present at an ectopic position.

21. The mouse of claim 19, wherein the nucleic acid sequence is present at an endogenous immunoglobulin locus.

22. The mouse of claim 19, wherein the nucleic acid sequence is present in the mouse genome at a position other than an endogenous immunoglobulin locus.

23. A mouse that expresses serum immunoglobulin, wherein greater than 80% of the serum immunoglobulin of the mouse comprises a human heavy chain variable domain and a cognate human light chain variable domain, wherein the human heavy chain variable domain is derived from a V_H gene segment repertoire consisting essentially of a single human V_H gene segment and/or polymorphic variants thereof.

24. A mouse comprising in its germline a replacement at an endogenous immunoglobulin heavy chain locus of all or substantially all endogenous V_H gene segments with single human V_H gene segment and/or polymorphic variants thereof.

25. The mouse of claim 24, further comprising a replacement at an endogenous immunoglobulin light chain locus of all or substantially all endogenous light chain V gene segments with one or more human light chain V gene segments.

26. The mouse of claim 25, further comprising one or more human light chain J gene segments operably linked to the human light chain V gene segments.

27. A cell or tissue derived from the mouse of claim 1, 19, 23, or 24.

28. A method of making a population of human antibodies comprising heavy chains derived from a single human V_H gene segment, the method comprising

(a) immunizing a mouse of claim 1 with an antigen of interest;

(b) allowing said mouse to mount an immune response with respect to the antigen of interest; and,

(c) identifying or isolating an immunoglobulin heavy chain variable region sequence of the non-human animal, wherein the antibody binds the antigen of interest.

29. The method of claim 28, wherein the immunoglobulin heavy chain variable region sequence is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to SEQ ID NO: 5.

30. The method of claim 28, wherein the immunoglobulin heavy chain variable region sequence comprises SEQ ID NO: 5.

31. The method of claim 28, wherein the immunoglobulin heavy chain variable region sequence is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to SEQ ID NO: 64.

32. The method of claim 28, wherein the immunoglobulin heavy chain variable region sequence comprises SEQ ID NO: 64.

33. A method for modifying an immunoglobulin heavy chain locus of a mouse, comprising:

(a) making a first modification of the mouse immunoglobulin heavy chain locus that results in a reduction or elimination of endogenous mouse ADAM6 activity in a male mouse; and,

(b) making a second modification of the mouse to restore ADAM6 activity to the mouse.

34. The method of claim 33, wherein the nucleic acid sequence in step (b) is present at an ectopic position.

35. The method of claim 33, wherein the first modification comprises the presence of one or more human immunoglobulin gene sequences.

36. The method of claim 33, wherein the first modification comprises the replacement of one or more sequences in the mouse immunoglobulin heavy chain locus with one or more human immunoglobulin gene sequences in the mouse immunoglobulin heavy chain locus.

37. The method of claim 36, wherein the first modification comprises the replacement of one or more endogenous V_H gene segments with a single human V_H gene segments in the mouse immunoglobulin heavy chain locus.

38. The method of claim 33, wherein the first modification comprises the replacement of an endogenous heavy chain variable gene sequence with a human heavy chain variable gene sequence in the mouse immunoglobulin heavy chain locus.

39. The method of claim 33, wherein the first and the second modification are made simultaneously.

40. A method for generating an antibody specific against an antigen comprising the steps of:

(a) immunizing a mouse of claim 1 with the antigen;

(b) isolating at least one cell from the mouse producing an antibody specific against the antigen; and

(c) culturing at least one cell producing an antibody of step b) and obtaining said antibody.

41. The method of claim 40, wherein the culturing in step (c) is performed on at least one hybridoma cell generated from the at least one cell obtained in step (b).

42. The method of claim 40, wherein the at least one cell of obtained in step (b) is derived from the spleen, a lymph node or bone marrow of the mouse from step (a).

43. The method of claim 40, wherein immunizing with the antigen of step (a) is carried out with protein, DNA, a combination of DNA and protein, or cells expressing the antigen.

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        35   40   45
Gly Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
    50   55   60
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<213> Homo sapiens

<400> 45
caggtccagc tggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cttctggagg caccttcagc agctatgcta tcagctgggt gcgacaggcc 120
cctggacaag ggcttgagtg gatgggaggg atcatcccta tctttggtac agcaaactac 180
gcacagaagt tccagggcag agtcacgatt accacggacg aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gaga 294

<210> 46
<211> 98
<212> PRT
<213> Homo sapiens

<400> 46
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20   25   30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Ile Thr Thr Asp Glu Ser Thr Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys

```
                85                  90                  95
Ala Arg


<210> 47
<211> 296
<212> DNA
<213> Homo sapiens

<400> 47
caggtgcagc tggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cttctggagg caccttcagc agctatgcta tcagctgggt gcgacaggcc 120
cctggacaag ggcttgagtg gatgggaggg atcatcccta tctttggtac agcaaactac 180
gcacagaagt tccagggcag agtcacgatt accgcggaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gagaga     296

<210> 48
<211> 98
<212> PRT
<213> Homo sapiens

<400> 48
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1               5                  10                  15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg


<210> 49
<211> 233
<212> DNA
<213> Homo sapiens

<400> 49
agaagcctgg gtcctcggtg aaggtctcct gcaaggcttc tggaggcacc ttcagcagct 60
atgctatcag ctgggtgcga caggcccctg gacaagggct tgagtggatg ggaaggatca 120
tccctatctt tggtacagca aactacgcac agaagttcca gggcagagtc acgattaccg 180
cggacgaatc cacgagcaca gcctacatgg agctgagcag cctgagatct gag        233

<210> 50
<211> 77
<212> PRT
<213> Homo sapiens

<400> 50
Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr
 1               5                  10                  15
```

```
Phe Ser Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly
            20                  25                  30
Leu Glu Trp Met Gly Arg Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr
        35                  40                  45
Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr
    50                  55                  60
Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu
65                  70                  75
```

<210> 51
<211> 296
<212> DNA
<213> Homo sapiens

<400> 51
caggtccagc tggtgcaatc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cttctggagg caccttcagc agctatacta tcagctgggt gcgacaggcc 120
cctggacaag ggcttgagtg gatgggaagg atcatcccta tccttggtac agcaaactac 180
gcacagaagt tccagggcag agtcacgatt accgcggaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gagaga 296

<210> 52
<211> 98
<212> PRT
<213> Homo sapiens

<400> 52
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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1               5                  10                  15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30
Thr Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45
Gly Arg Ile Ile Pro Ile Leu Gly Thr Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg
```

<210> 53
<211> 296
<212> DNA
<213> Homo sapiens

<400> 53
caggtgcagc tggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cttctggagg caccttcagc agctatgcta tcagctgggt gcgacaggcc 120
cctggacaag ggcttgagtg gatgggaagg atcatcccta tccttggtat agcaaactac 180
gcacagaagt tccagggcag agtcacgatt accgcggaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gagaga 296

<210> 54
<211> 98

<212> PRT
<213> Homo sapiens

<400> 54
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20   25   30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg

<210> 55
<211> 296
<212> DNA
<213> Homo sapiens

<400> 55
caggtccagc tggtgcagtc tggggctgag gtgaagaagc ctgggtcctc agtgaaggtc 60
tcctgcaagg cttctggagg caccttcagc agctatgcta tcagctgggt gcgacaggcc 120
cctggacaag ggcttgagtg gatgggaggg atcatcccta tccttggtat agcaaactac 180
gcacagaagt tccagggcag agtcacgatt accgcggaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gagaga   296

<210> 56
<211> 98
<212> PRT
<213> Homo sapiens

<400> 56
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20   25   30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Gly Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg

<210> 57
<211> 296
<212> DNA
<213> Homo sapiens

<400> 57
caggtccagc tggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cttctggagg caccttcagc agctatgcta tcagctgggt gcgacaggcc 120
cctggacaag ggcttgagtg gatgggaagg atcatcccta tccttggtac agcaaactac 180
gcacagaagt tccagggcag agtcacgatt accgcggacg aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gagaga 296

<210> 58
<211> 98
<212> PRT
<213> Homo sapiens

<400> 58
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20   25   30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Arg Ile Ile Pro Ile Leu Gly Thr Ala Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg

<210> 59
<211> 296
<212> DNA
<213> Homo sapiens

<400> 59
caggtccagc tggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cttctggagg caccttcagc agctatgcta tcagctgggt gcgacaggcc 120
cctggacaag ggcttgagtg gatgggaggg atcatcccta tctttggtac agcaaactac 180
gcacagaagt tccagggcag agtcacgatt accgcggacg aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gagaga 296

<210> 60
<211> 98
<212> PRT
<213> Homo sapiens

<400> 60
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20   25   30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65   70   75   80

```
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg
```

<210> 61
<211> 296
<212> DNA
<213> Homo sapiens

<400> 61
caggtccagc tggtgcagtc tggggctgag gtgaagaagc ctgggtcctc agtgaaggtc 60
tcctgcaagg cttctggagg caccttcagc agctatgcta tcagctgggt gcgacaggcc 120
cctggacaag ggcttgagtg gatgggaggg atcatcccta tctttggtac agcaaactac 180
gcacagaagt tccagggcag agtcacgatt accgcggacg aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gagaga 296

<210> 62
<211> 98
<212> PRT
<213> Homo sapiens

<400> 62
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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1               5                  10                  15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60
Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg
```

<210> 63
<211> 305
<212> DNA
<213> Homo sapiens

<400> 63
gagcccactc ccaggtgcag ctggtgcagt ctggggctga ggtgaagaag cctggggcct 60
cagtgaaggt ctcctgcaag gcttctggat acaccttcac cggctactat atgcactggg 120
tgcgacaggc ccctggacaa gggcttgagt ggatgggacg gatcaaccct aacagtggtg 180
gcacaaacta tgcacagaag tttcagggca gggtcaccag taccagggac acgtccatca 240
gcacagccta catggagctg agcaggctga gatctgacga cacggtcgtg tattactgtg 300
cgaga 305

<210> 64
<211> 98
<212> PRT
<213> Homo sapiens

<400> 64
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20   25   30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Arg Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Ser Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Val Val Tyr Tyr Cys
                85   90   95
Ala Arg

<210> 65
<211> 307
<212> DNA
<213> Homo sapiens

<400> 65
gagcccactc ccaggtgcag ctggtgcagt ctggggctga ggtgaagaag cctggggcct 60
cagtgaaggt ctcctgcaag gcttctggat acaccttcac cggctactat atgcactggg 120
tgcgacaggc ccctggacaa gggcttgagt ggatgggatg gatcaaccct aacagtggtg 180
gcacaaacta tgcacagaag tttcagggca gggtcaccat gaccagggac acgtccatca 240
gcacagccta catggagctg agcaggctga gatctgacga cacggccgtg tattactgtg 300
cgagaga   307

<210> 66
<211> 98
<212> PRT
<213> Homo sapiens

<400> 66
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20   25   30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg

<210> 67
<211> 307
<212> DNA
<213> Homo sapiens

<220>

<221> variation
<222> (124)...(124)
<223> n=a, g, t, or c

<400> 67
gagcccactc ccaggtgcag ctggtgcagt ctggggctga ggtgaagaag cttggggcct 60
cagtgaaggt ctcctgcaag gcttctggat acaccttcac cggctactat atgcactggg 120
tgcnacaggc ccctggacaa gggcttgagt ggatgggatg gatcaaccct aacagtggtg 180
gcacaaacta tgcacagaag tttcagggca gggtcaccat gaccagggac acgtccatca 240
gcacagccta catggagctg agcaggctga gatctgacga cacggccgtg tattactgtg 300
cgagaga 307

<210> 68
<211> 98
<212> PRT
<213> Homo sapiens

<220>
<221> VARIANT
<222> (38)...(38)
<223> Xaa=any amino acid

<400> 68
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Leu Gly Ala
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
20 25 30
Tyr Met His Trp Val Xaa Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
50 55 60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg

<210> 69
<211> 294
<212> DNA
<213> Homo sapiens

<400> 69
caggtgcagc tggtgcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60
tcctgcaagg cttctggata caccttcacc ggctactata tgcactgggt gcgacaggcc 120
cctggacaag ggcttgagtg gatgggatgg atcaacccta acagtggtgg cacaaactat 180
gcacagaagt ttcagggctg ggtcaccatg accagggaca cgtccatcag cacagcctac 240
atggagctga gcaggctgag atctgacgac acggccgtgt attactgtgc gaga 294

<210> 70
<211> 98
<212> PRT
<213> Homo sapiens

<400> 70
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala

```
 1               5                  10                  15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45
Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60
Gln Gly Trp Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg
```

<210> 71
<211> 346
<212> DNA
<213> Homo sapiens

<400> 71
aggatgtggg ttttcacact gtgtctctcg cacagtaata cacgaccgtg tcgtcagatc 60
tcagcctgct cagctccatg taggctgtgc tgatggacgt gtccctggtc atggtgaccc 120
tgccctgaaa cttctgtgca tagtttgtgc caccactgtt agggttgatc cgtcccatcc 180
actcaagccc ttgtccaggg gcctgtcgca cccagtgcat atagtagccg gtgaaggtgt 240
atccagaagc cttgcaggag accttcactg aggccccagg cttcttcacc tcagccccag 300
actgcaccag ctgcacctgg gagtggacac ctgtggagac tcgcga 346

<210> 72
<211> 98
<212> PRT
<213> Homo sapiens

<400> 72
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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1               5                  10                  15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45
Gly Arg Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Val Val Tyr Tyr Cys
                85                  90                  95
Ala Arg
```

<210> 73
<211> 14781
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic

<400> 73
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tctgcctcca cgagaatcac catgtccttt ctcaagactg tgttctgtgc agtgccctgt 120
cagtggaaat ctggagagca tgcttccatg agcttgtgag tagtatatct agtaagccat 180
ggctttgtgt taatggtgat gttctacata tcagttctct ggcttaataa tgaggtgatg 240
attctatgtt cctgtaacgc ttcctcaact gggtcctaag tctttcttca ctccatctat 300
tcctctaagg aatgatcctg aaaatcccat cacaaactat aggagatggg aaccatcaaa 360
aaacacagtg acaaagaggt gggaacgcat cagggttcag gaaccatatt ttaaaaagat 420
atcgtaaata acttcttaaa agagatatag acaaatctcc attaatacgg agaccagagg 480
cctaaggcta agaaccaatg gtggctcaag gtctcctgct acccgaggag caaacgtaga 540
gcagtttcta atgatttatt taaaatatag aatcaaaagt accagtttgc aattttgaaa 600
gatttatttc agcaatgcaa caacatcagg tggtgccgag tccaacacgt cttatgtccc 660
atgatataaa caaaggccat ccagaactgt ggactggagt tctaccttgt cccctaatga 720
cattcagatt ttttttccat tctctttatc ttagaggaga caggggggcta actcatttta 780
cttgtccttt gcttgttctt gccaagaacg taaagcagct tgcaagtctt caaacctaaa 840
tatcttagta actcctacac gagtggcaat gccaaagagc agtgcaacaa agaggaagta 900
aatacgacca aagagtattc ttaaatacac tactggctct aggttctgtt ttattatgcg 960
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tgtttctgtc ccacgatgtt catcaagccc aaagcaggta accccagaga taaccgattg 1260
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agcacttaca ccaaagattt ccatgcattg tatgttgcga tcagtgcagt tacctttata 1500
gcagtaaccc tcttctgagc atggtgtccc atcttgcaga taagtgtcat ctgggcaaat 1560
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tgtcccagaa ggactgtaag tgcaatttgc acagcataat tctttatcac aaatgctacc 1680
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tatgttgctt atctctgcaa gggaacaatt gctgaaagca tctgttaatt gaggattttt 1920
gaacatgatg caggtgttcc ttctctggca gatacagtac ccctcatcat gttttaggcc 1980
taaactcctt ccaacacgat tggttattat aatagataaa aataaaggat ttcgaccatg 2040
ttgaccaaga caaattaggg ctgagggaga acatatactc ctctcagctg gattaacagc 2100
atcatctcct ggcgaattct tgttaattat agctcctgca tcaggcctaa aatgagcata 2160
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tggatcagcc tcggtataca cagtcatgag aaagacatag taccgcatat gaagattggt 2280
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gtgtctgggc caaatgagtg tctttctccg caggtggatg atatgtctct ggccccgaaa 2820
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gactggaacc aggaggagca gccagagtgc aaatagcaag aggaggaccc tggggaccac 3000
aggtctttcc actagcctca tgccccaggt cagagataac atcctgggtg gagctaactc 3060
cctctgctgt ggccactgcc tggtctagaa aatactgaca gaggactaaa aacctcctca 3120
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caggaattga gtctgaatgt gttagctgag gttgaggtta aatattgtca aaagggatgt 3240
ctataaatgt gcctggacaa gaaaagtcag aagcagcaag gagtgtctct gacaggctca 3300
atcctttctt ttctttttct gaagttcaaa atatcatttc cacgtgaatg tatttggttc 3360
ccagtgtgac tctgggtctc tttctaggag tcaatatttc tttatatctt ggctcatgtt 3420

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