HUMANIZED RODENTS THAT EXPRESS HEAVY CHAINS CONTAINING V_L DOMAINS

FIELD OF INVENTION

[0001] Genetically modified non-human fertile animals that express human immunoglobulin-like binding proteins comprising an immunoglobulin heavy chain constant region fused with an immunoglobulin light chain variable domain are provided, as well as binding proteins having an immunoglobulin light chain variable domain fused to a light chain constant domain and an immunoglobulin light chain variable domain fused to a heavy chain constant domain. Genetically modified mice, cells, embryos, and tissues that comprise a nucleic acid sequence that encodes a functional ADAM6 protein are described, wherein the mice, cells, embryos, and tissues comprise human immunoglobulin light chain gene segments operably linked to one or more non-human immunoglobulin heavy chain constant genes. Modifications include human and/or humanized immunoglobulin loci. Mice that comprise ADAM6 function are described, including mice that comprise an ectopic nucleic acid sequence that encodes an ADAM6 protein. Genetically modified male mice that comprise a genetic modification of an endogenous mouse immunoglobulin V_H region locus, and that further comprise ADAM6 activity are described, including mice that comprise an ectopic nucleic acid sequence that restores or maintains fertility to the male mouse. Exemplary fertility is fertility that is comparable to wild-type mice.

[0002] Genetically modified non-human fertile animals that comprise a deletion or a modification of an endogenous ADAM6 gene or homolog or ortholog thereof, and that comprise a genetic modification that restores ADAM6 (or homolog or ortholog thereof) function in whole or in part are provided, wherein the non-human animals express a human immunoglobulin light chain variable sequence in the context of a heavy chain constant sequence. Cells expressing such binding proteins, rodents (*e.g.*, mice) that make them, and related methods and compositions are also provided.

[0003] Genetically engineered animals that express antibodies comprising light chain variable regions fused with heavy chain constant regions, wherein the non-human animals lack a functional endogenous ADAM6 gene but retain ADAM6 function, are described, including rodents (*e.g.,* 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 plurality of human V_L, J_L and optionally D_H gene segments or a combination thereof, and that further comprise ADAM6 function are described, including mice that comprise an ectopic nucleic acid sequence that restores fertility to a male mouse. The genetically modified mice express antibodies that

lack heavy chain variable domains and instead comprise variable domains comprising rearranged light chain gene segments.

[0004] Genetically modified rodents (*e.g.,* 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 comprising a human light chain variable domain. 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 antigens are provided.

BACKGROUND

[0005] Over the last two decades, pharmaceutical applications for antibodies fueled a great deal of research into making antibodies that are suitable for use as human therapeutics. Early antibody therapeutics, based on mouse antibodies, were not ideal as human therapeutics because repeatedly administering mouse antibodies to humans results in immunogenicity problems that can confound long-term treatment regimens. Solutions based on humanizing mouse antibodies to make them appear more human and less mouse-like were developed. Methods for expressing human immunoglobulin sequences for use in antibodies followed, mostly based on *in vitro* expression of human immunoglobulin libraries in phage, bacteria, or yeast. Finally, attempts were made to make useful human antibodies from human lymphocytes *in vitro*, in mice engrafted with human hematopoietic cells, and in transchromosomal or transgenic mice with disabled endogenous immunoglobulin loci.

[0006] 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.

[0007] Transgenic mice that contain fully human antibody transgenes contain randomly inserted transgenes that contain unrearranged human immunoglobulin heavy chain variable sequences (V, D, and J sequences) linked to human heavy chain constant sequences, and

unrearranged human immunoglobulin light chain variable sequences (V and J) linked to human light chain constant sequences. The mice therefore generate rearranged antibody genes from loci other than endogenous loci, where the rearranged antibody genes are fully human. In general, the mice contain human heavy chain sequences and human κ light chain sequences, although mice with at least some human λ sequences have also been reported. The transgenic mice generally have damaged and nonfunctional endogenous immunoglobulin loci, or knockouts of endogenous immunoglobulin loci, so that the mice are incapable of rearranging human antibody sequences at an endogenous immunoglobulin locus. The vagaries of such transgenic mice render them less than optimal for generating a sufficiently diverse human antibody repertoire in mice, likely due at least in part to a suboptimal clonal selection process that interfaces fully human antibody molecules within an endogenous selection system and deleterious effects from changes to the endogenous genetic makeup of such mice.

[0008] There remains a need in the art for making improved genetically modified non-human animals that are useful in generating immunoglobulin sequences, including human antibody sequences, and that are useful in generating a diverse repertoire of immunoglobulin-like molecules that exhibit diversity apart from traditional antibody molecules, while at the same time reducing or eliminating deleterious changes that might result from the genetic modifications. There also remains a need for non-human animals that are capable of rearranging immunoglobulin gene segments to form useful rearranged immunoglobulin genes, including human immunoglobulin light chain variable domains in the context of heavy chain constant domains that are cognate with human immunoglobulin light chain variable domains in the context of light chain constant domains, or that are capable of making proteins from altered immunoglobulin loci, including loci that contain a sufficiently diverse collection of human light chain variable gene segments. There remains a need for non-human animals that can generate immunoglobulin-like binding proteins, wherein the binding proteins comprise human immunoglobulin light chain variable domains linked to heavy chain constant domains.

SUMMARY OF INVENTION

[0009] Genetically modified non-human animals having immunoglobulin loci are provided, wherein the immunoglobulin loci comprise a plurality of human light chain variable (V_L) gene segments operably linked to one or more non-human constant regions, *e.g.,* human Vκ and Jκ or human Vλ and Jλ, and in various embodiments the loci lack a sequence that encodes an endogenous functional ADAM6 protein. The non-human animals include rodents, *e.g.,* mice and rats.

[0010] Loci are provided that are capable of rearranging and forming a gene encoding a light chain variable domain that is derived from a rearrangement involving a human light chain Vκ or Vλ gene segment and a human Jκ or Jλ gene segment and in various embodiments, additionally a D_H gene segment, wherein in various embodiments the loci lack an endogenous functional ADAM6 gene or functional fragment thereof.

[0011] Modified immunoglobulin loci include loci that lack an endogenous functional ADAM6 gene and comprise human immunoglobulin sequences are provided, *e.g.,* a human V_L 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 V and/or a J segment). Modified loci that comprise multiple V_L gene segments 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 V_L gene segments, operably linked with one or more D_H segments and/or one or more J_L or J_H segments, operably linked to a non-human immunoglobulin constant sequence, *e.g.*, a rodent (*e.g.*, mouse or rat) or human sequence, are provided. Non-human animals comprising such humanized loci are also provided, wherein the non-human animals exhibit wild-type fertility.

[0012] Non-human animals that comprise an immunoglobulin heavy chain variable locus (*e.g.,* on a transgene or as an insertion or replacement at an endogenous non-human animal heavy chain variable locus) that comprises a plurality of human V_L gene segments operably linked to a human D and/or human J gene segment are provided. In various embodiments, the plurality of human V_L gene segments are operably linked to one or more human D and/or one or more human 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 at least one human V_L segment, a D_H gene segment, or a J_L 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.

[0013] 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 plurality of human V_L gene segments operably linked to a D_H and J segment or a

J_L gene 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 are also provided.

[0014] 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 heavy chain sequences that comprise human V_L gene segments rearranged and in operable linkage with immunoglobulin heavy chain constant regions, *e.g.,* V_L, and one or more D_H and J segments or one or more J_L segments. Methods for making human immunoglobulin sequences in non-human animals, tissues, and cells are provided, wherein the human immunoglobulin sequences bind an antigen of interest.

[0015] In one aspect, nucleic acid constructs, cells, embryos, rodents (*e.g.*, mice), and methods are provided for making rodents (*e.g.*, mice) that comprise a modification that results in a nonfunctional endogenous rodent (*e.g.*, mouse) ADAM6 protein or ADAM6 gene (*e.g.*, a knockout of or a deletion in an endogenous ADAM6 gene), wherein the rodents (*e.g.*, mice) comprise a nucleic acid sequence that encodes an ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a male rodent of the same kind (*e.g.*, 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. In one embodiment, the mice comprise an ectopic nucleotide sequence that encodes one or more rodent ADAM6 proteins, wherein the one or more proteins comprise SEQ ID NO: 1 or SEQ ID NO: 2 or a fragment thereof that is functional in the mice.

[0016] In various aspects, the sequence that encodes ADAM6 activity is contiguous with a human immunoglobulin sequence. In various aspects, the sequence that encodes ADAM6 activity is contiguous with a non-human immunoglobulin sequence. In various aspects, the sequence is present on the same chromosome as the endogenous non-human immunoglobulin heavy chain locus of the non-human animal. In various aspects, the sequence is present on a different chromosome than the immunoglobulin heavy chain locus of the non-human animal.

[0017] Genetically modified non-human animals are described that comprise a modification that maintains activity of an ADAM6 gene or homolog or ortholog thereof, wherein the modification includes insertion of one or more human immunoglobulin light chain gene segments upstream of a non-human immunoglobulin heavy chain constant region, and the non-human animals further comprise modifications that enable them to express human immunoglobulin light chain variable regions cognate with human immunoglobulin light chain

variable regions. In various aspects, the human immunoglobulin light chain variable regions are expressed in the context of light and heavy chain constant regions.

[0018] In various aspects, the insertion of one or more human immunoglobulin light chain gene segments is performed 3' or downstream of the ADAM6 gene of the non-human animal. In various aspects, the insertion of one or more human immunoglobulin light chain gene segments is performed in a manner such that the ADAM6 gene(s) of the non-human animal is not disrupted, deleted and/or functionally silenced such that the ADAM6 activity of the non-human animal is at the same or comparable level as in a non-human animal that does not contain such an insertion. Exemplary disruptions, deletions and/or functionally silencing modifications include any modification that results in a reduction, elimination and/or loss of activity of the ADAM6 protein(s) encoded by the ADAM6 gene(s) of the non-human animal.

[0019] In one aspect, nucleic acid constructs, cells, embryos, mice, and methods are provided for making mice that comprise a modification of an endogenous 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 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.

[0020] 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 functional fragment thereof is located at an ectopic position as compared to an endogenous ADAM6 gene of a wild type mouse.

[0021] In one aspect, methods are provided for making mice that comprise a modification of an endogenous immunoglobulin locus, wherein the mice comprise an ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a male mouse. In various embodiments, the modification comprises an insertion of one or more human V_L gene segments at the endogenous immunoglobulin locus.

[0022] In one aspect, methods are provided for making mice that comprise a genetic modification of a heavy chain immunoglobulin locus, wherein application of the methods result in male mice that comprise a modified heavy chain immunoglobulin 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.

[0023] In one aspect, methods are provided for making mice that comprise a genetic modification of an immunoglobulin heavy chain locus and an immunoglobulin light chain locus, wherein application of the methods to modify the heavy chain locus result in male mice that 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.

[0024] In one embodiment, the genetic modification comprises replacing endogenous immunoglobulin heavy chain variable loci with immunoglobulin light chain variable loci of another species (*e.g.*, a non-mouse species). In one embodiment, the genetic modification comprises insertion of immunoglobulin light chain variable loci of another species (*e.g.,* a non-mouse species) 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.

[0025] In one embodiment, the genetic modification comprises inactivation of an endogenous non-human immunoglobulin heavy chain variable locus in whole or in part, wherein the inactivation does not result in a loss of endogenous ADAM6 function. Inactivation may include replacement or deletion of one or more endogenous non-human gene segments resulting in an endogenous non-human immunoglobulin heavy chain locus that is substantially incapable of rearrangement to encode a heavy chain of an antibody that comprises endogenous non-human 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). Other exemplary modifications include disabling the operable linkage between the non-

human immunoglobulin variable gene segments and the non-human immunoglobulin constant regions.

[0026] In one embodiment, the genetic modification comprises inserting into the genome of the non-human animal a DNA fragment containing one or more human V_L gene segments and one or more human J_L gene segments, and optionally one or more human D_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 in the genome of the non-human animal to form a sequence that encodes a light chain variable domain operably linked to a heavy chain constant region. In one embodiment, the species is human. In one embodiment, the genetic modification comprises insertion of one or more human immunoglobulin light chain gene segments downstream or 3' of an endogenous ADAM6 gene of the non-human animal such that ADAM6 activity (*e.g.*, expression and/or function of an encoded protein) is the same or comparable to a non-human animal that does not comprise the insertion.

[0027] 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.

[0028] In one embodiment, the genetic modification comprises replacing endogenous immunoglobulin heavy chain variable loci with immunoglobulin light chain variable loci, *e.g.*, one or more light chain variable (V_L) gene segments, one or more heavy chain diversity (D_H) gene segments and one or more joining (J) gene segments, or one or more light chain joining (J_L) gene segments of another species (*e.g.*, a non-mouse species). In one embodiment, the genetic modification comprises insertion of a single orthologous immunoglobulin light chain variable loci V_L gene segment, at least one D_H gene segment and at least one J gene segment, or at least one J_L gene segment into endogenous immunoglobulin heavy chain variable loci. In a specific embodiment, the species is human. In one embodiment, the genetic modification comprises a 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 alterations that result in a heavy chain locus that is not in operable linkage with one or more endogenous constant regions.

[0029] In one embodiment, the genetic modification comprises inserting into the genome of the mouse a DNA fragment containing one or more human V_L gene segments, one or more J gene segments, and optionally one or more D 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 various embodiments, the J gene segments include J_H or J_L gene segments. In one embodiment, the DNA fragment is capable of undergoing rearrangement to form a sequence that encodes a heavy chain of an antibody, wherein the heavy chain comprises a rearranged human light chain variable gene segment fused to a heavy chain constant region. In one embodiment, the genetic modification comprises insertion of at least six, at least 16, at least 30, or at least 40 or more human V_L gene segments, and at least one or at least 5 human J_L gene segments into the genome of the mouse. In a specific embodiment, the species is human and the gene segments are human κ light chain gene segments. 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.

[0030] 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.

[0031] 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.

[0032] 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.

[0033] 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.

[0034] 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 at least one 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 at least one human V_L and at least one human J_L gene segment. In another specific embodiment, the heavy chain locus comprises at least one human V_L, at least one human D_H, and at least one human J_L. In another specific embodiment, the heavy chain locus comprises at least one human V_L, at least one human D_H, and at least one human J_H gene segment. In another specific embodiment, the heavy chain locus comprises at least one human V_L and at least one human J_L gene segment. In another specific embodiment, the heavy chain locus comprises at least one human V_L and at least one human J_H gene segment. In another specific embodiment, the heavy chain locus comprises six human $V\kappa$ and five human $J\kappa$ gene segments. In another specific embodiment, the heavy chain locus comprises 16 human $V\kappa$ and five human $J\kappa$ gene segments. In another specific embodiment, the heavy chain locus comprises 30 human $V\kappa$ and five human $J\kappa$ gene

segments. In another specific embodiment, the heavy chain locus comprises 40 human Vκ and five human Jκ gene segments.

[0035] 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 ROSA 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.

[0036] 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.

[0037] 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 at least one human V_L and at least one human J_L gene segment. In a specific embodiment, the heavy chain locus comprises at least six and up to 40 human Vκ gene segments, and five human Jκ 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.

[0038] 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.

[0039] 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.

[0040] 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.

[0041] 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 four or six 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.

[0042] 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.

[0043] 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 six hours at an efficiency that is about equal to sperm from a wild-type mouse.

[0044] 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.

[0045] 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 human heavy chain sequence contiguous with a human light chain sequence. In one embodiment, the sequence comprises one or more V gene segments, one or more D gene segments, and one or more J gene segments; in one embodiment, the sequence comprises one or more V gene segments and one or more J gene segments. In one embodiment, the one or more V, D, and J gene segments, or one or more V and J gene segments, are unrearranged. In one embodiment, the one or more V, D, and J gene segments, or one or more V and J gene segments, are rearranged. In one embodiment, following rearrangement of the one or more V, D, and J gene segments, or one or more V and J 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.

[0046] In one aspect, mice are provided that express a human immunoglobulin heavy chain variable region or functional fragment thereof from an endogenous immunoglobulin heavy chain locus, wherein the mice comprise an ADAM6 activity that is functional in a male mouse. In one embodiment, the heavy chain locus comprises one or more human V_L gene segments and one or more J_L gene segments, and optionally one or more D_H gene segments. In one embodiment, the heavy chain locus comprises at least six human $V\kappa$ gene segments and five human $J\kappa$ gene segments. In one embodiment, the heavy chain locus comprises at least 16 human $V\kappa$ gene segments and five human $J\kappa$ gene segments. In one embodiment, the heavy chain locus comprises at least 30 human $V\kappa$ gene segments and five human $J\kappa$ gene segments. In one embodiment, the heavy chain locus comprises at least 40 human $V\kappa$ gene segments and five human $J\kappa$ gene segments.

[0047] In one aspect, mice are provided that express a human immunoglobulin light chain variable region or functional fragment thereof from an endogenous immunoglobulin heavy chain locus, wherein the mice comprise an ADAM6 activity that is functional in a male mouse.

[0048] 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.

[0049] 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.

[0050] 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 ADAM6 allele, *e.g.,* 3' of a final V gene segment sequence and 5' of an initial J gene segment.

[0051] 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 is different from that of the endogenous ADAM6 allele, *e.g.,* 5' of the 5'-most V gene segment sequence of a V gene locus.

[0052] 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 V gene segment and/or an immunoglobulin J gene segment. In a specific embodiment, the immunoglobulin V and J gene segments are human gene segments. In one embodiment, the immunoglobulin V and J gene segments are human gene segments, and the sequence encoding the mouse ADAM6 or ortholog or homolog or fragment thereof functional in a mouse is between the human V and J gene segments; in one embodiment, the mouse comprises two or more human V gene segments, and the sequence is at a position 5' of the 5'-most human V gene segment; in one embodiment, the mouse comprises two or more human V gene segments, and the sequence is at a position between the final V gene segment and the penultimate V gene segment; in one embodiment, the mouse comprises a plurality of human V gene segments, and the sequence is at a position upstream of the 5'-most human V gene segment; in one embodiment, the mouse further comprises a D gene segment, and the sequence is at a position following the 3'-most V gene segment and a 5'-most D gene segment; in one embodiment, the sequence is at a position between a V gene segment and a J gene segment.

[0053] In one embodiment, the human V gene segments are light chain V gene segments. In a specific embodiment, the light chain V gene segments are Vκ gene segments. In another specific embodiment, the light chain V gene segments are Vλ gene segments. In one embodiment, the J gene segment is selected from a J_H and a J_L gene segment. In a specific embodiment, the J_L gene segment is a Jκ gene segment. In another specific embodiment, the J_L gene segment is a Jλ gene segment.

[0054] 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 variable region of an antibody, wherein the variable region comprises or is derived from an endogenous non-human gene segment. In a specific embodiment, the endogenous locus is positioned at a location in the genome of the male mouse that renders the heavy chain gene segments of the locus incapable of encoding a heavy chain variable region 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.

[0055] 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.

[0056] In one aspect, a male mouse is provided that comprises a functional endogenous ADAM6 gene and a modification to an endogenous immunoglobulin heavy chain locus. In one embodiment, the modification is made downstream, or 3' of an endogenous ADAM6 gene or locus. In one embodiment, the modification is a replacement of one or more endogenous immunoglobulin heavy chain gene segments with one or more human immunoglobulin light chain gene segments. In one embodiment, the modification is an insertion of one or more human immunoglobulin light chain gene segments upstream of an endogenous immunoglobulin heavy chain constant region gene.

[0057] In one aspect, mice are provided that comprise a genetic modification that reduces endogenous 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. In various embodiments, the ADAM6 or ortholog or homolog or functional fragment thereof comprises a nucleic acid sequence that encodes an ADAM6 protein set forth in SEQ ID NO: 1, SEQ ID NO: 2, or a combination thereof.

[0058] 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 mouse is incapable of expressing an antibody comprising an endogenous immunoglobulin heavy chain gene segment. 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 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.

[0059] 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.

[0060] 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.

[0061] 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.*, extra-chromosomally, *e.g.*, in an expression construct, a vector, a YAC, a trans-chromosome, *etc.*).

[0062] In one aspect, genetically modified mice and cells are provided that comprise a modification of an endogenous heavy chain immunoglobulin locus, wherein the mice express at least a portion of a immunoglobulin light 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 an 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 rearranging to encode a heavy chain variable region of an antibody.

[0063] 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 heavy chain allele comprises a first modification that reduces or

eliminates expression of a functional ADAM6 from the first heavy chain allele, and the mouse comprises a second 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 heavy chain allele.

[0064] In various embodiments, the modification is the insertion of one or more human immunoglobulin light chain gene segments upstream, or 5', of an endogenous immunoglobulin heavy chain constant region gene. In various embodiments, the modification maintains the endogenous ADAM6 gene located at the endogenous immunoglobulin heavy chain locus.

[0065] 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).

[0066] 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 human V, D, and/or J gene segment, a replacement with a camelid V, D, and/or J gene segment, a replacement with a humanized or camelized V, D, and/or J 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 heavy chain V, D, and/or J gene segments and a replacement with one or more light chain V and/or J gene segments (*e.g.*, a human light chain V and/or J gene segment) at the heavy chain locus.

[0067] 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.

[0068] 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 an endogenous ADAM6 gene in the germline of the mouse. In a specific embodiment, the endogenous ADAM6 gene is juxtaposed by mouse immunoglobulin heavy chain gene segments.

[0069] 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 sequence 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 or activity results from expression of an ectopic ADAM6 at the second immunoglobulin heavy chain allele.

[0070] 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 immunoglobulin heavy chain locus. In one embodiment, the modified immunoglobulin sequence comprises a human light chain variable region sequence at an endogenous immunoglobulin heavy chain locus. In one embodiment, the human light chain variable region sequence replaces an endogenous heavy chain variable sequence at the endogenous immunoglobulin heavy chain locus. In one embodiment, the modified immunoglobulin sequence comprises a human κ light chain variable region sequence at an endogenous immunoglobulin heavy chain locus. In one embodiment, the modified immunoglobulin sequence comprises a human λ light chain variable region sequence at an endogenous immunoglobulin heavy chain locus.

[0071] In one aspect, a mouse incapable of expressing a functional endogenous ADAM6 from an endogenous 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.

[0072] 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 ADAM6 sequence or functional fragment thereof. In one embodiment, the endogenous 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).

[0073] 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 an ubiquitin promoter.

[0074] 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.

[0075] 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.

[0076] 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.

[0077] 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.

[0078] In one embodiment, the mouse ADAM6 promoter is a promoter of SEQ ID NO: 4. In a specific embodiment, the mouse ADAM6 promoter comprises the nucleic acid sequence of SEQ ID NO: 4 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: 4 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.

[0079] In one embodiment, the nucleic acid sequence comprises SEQ ID NO: 4 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: 4 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.

[0080] In one embodiment, the mouse ADAM6 promoter is a promoter of SEQ ID NO: 5. In a specific embodiment, the mouse ADAM6 promoter comprises the nucleic acid sequence of SEQ ID NO: 5 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: 5 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.

[0081] In one embodiment, the nucleic acid sequence comprises SEQ ID NO: 5 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: 5 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.

[0082] In various embodiments, the ectopic nucleic acid sequence that confers upon the mouse ADAM6 function encodes one or more ADAM6 proteins, wherein the one or more ADAM6 proteins comprise SEQ ID NO: 1, SEQ ID NO: 2 or a combination thereof.

[0083] In various embodiments, the ectopic nucleic acid sequence comprises a sequence selected from SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5, wherein the ectopic nucleic acid sequence confers upon the mouse ADAM6 function through one or more ADAM6 proteins encoded by the ectopic nucleic acid sequence.

[0084] In one embodiment, the nucleic acid sequence is any sequence encoding an ADAM6 gene or homolog or ortholog or functional fragment thereof that when placed into or maintained in a mouse yields a level of fertility that is the same or comparable to a wild-type

mouse. An exemplary level of fertility may be demonstrated by the ability of a male mouse to produce a sperm cell that is capable of traversing a female mouse oviduct in order to fertilize a mouse egg.

[0085] 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 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.

[0086] 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 V_H gene segment with a human V_L 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. In one embodiment, the human V_L gene segment is a $V\kappa$ gene segment. In one embodiment, the V_L gene segment is a $V\lambda$ gene segment.

[0087] In one embodiment, the mouse further comprises a human J_L gene segment, and the ectopic nucleotide sequence that encodes a mouse ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a male mouse is positioned between a human V_L gene segment and the human J_L gene segment. In one embodiment, the mouse comprises a one or more human V_L gene segments and one or more human V_L gene segments and the ectopic nucleotide sequence that encodes a mouse ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a male mouse is positioned upstream (or 5') of the one or more human V_L gene segments. In a specific embodiment, the human V_L and J_L gene segments are $V\kappa$ and $J\kappa$ gene segments.

[0088] 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.

[0089] In one embodiment, the mouse comprises a replacement of all or substantially all endogenous V_H gene segments with a nucleotide sequence encoding one or more human V_L gene segments, and the ectopic nucleotide sequence encoding the mouse ADAM6 protein is within, or directly adjacent to, the nucleotide sequence encoding the one or more human V_L gene segments. In one embodiment, the mouse further comprises a replacement of one or more endogenous D_L gene segments with one or more human V_L and/or human J_L 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_L 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 human V_L and J_L gene segments, wherein the mouse comprises an ectopic sequence encoding a mouse ADAM6 protein. In one embodiment, the mouse comprises an insertion of one or more human V_L and J_L gene segments at an endogenous immunoglobulin heavy chain locus, wherein the mouse comprises an ADAM6 gene that is functional in the mouse. In a specific embodiment, the human V_L and J_L gene segments are Vκ and Jκ gene segments. In a specific embodiment, the ectopic sequence encoding the mouse ADAM6 protein is placed between the penultimate 3'-most V_L gene segment of the human V_L gene segments present, and the ultimate 5'-most J_L gene segment of the human J_L gene segments present. In a specific embodiment, the ectopic sequence encoding the mouse ADAM6 protein is placed upstream (or 5') of the 5'-most V_L gene segment of the human V_L gene segments present. In a specific embodiment, the mouse comprises a deletion of all or substantially all mouse V_H gene segments, and a replacement with at least 40 human V_L gene segments, and the ectopic nucleotide sequence encoding the mouse ADAM6 protein is placed downstream of human Vκ4-1 gene segment and upstream of human Jκ1 gene segment. In a specific embodiment, the mouse comprises a deletion of all or substantially all mouse V_H gene segments, and a replacement with at least 40 human V_L gene segments, and the ectopic nucleotide sequence encoding the mouse ADAM6 protein is placed upstream of a human Vκ2-40 gene segment.

[0090] In a specific embodiment, the mouse comprises a replacement of all or substantially all endogenous V_H gene segments with a nucleotide sequence encoding one or more human V_L gene segments, and the ectopic nucleotide sequence encoding the mouse ADAM6 protein is within, or directly adjacent to, the nucleotide sequence encoding the one or more human V_L gene segments.

[0091] In one embodiment, the V_L gene segments are Vκ gene segments. In one embodiment, the V_L gene segments are Vλ gene segments.

[0092] 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 extra-chromosomally in the mouse.

[0093] In one aspect, a mouse is provided that comprises a modification of an endogenous heavy chain immunoglobulin 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 light chain V, J, and optionally a D gene 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.

[0094] In one embodiment, the human light chain V and/or J sequence is selected from a human Vκ, Vλ, Jκ and Jλ sequence.

[0095] 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 human V_L and JL gene segments, and optionally human D_H gene segments. In one embodiment, the one or more human immunoglobulin gene segments includes a human Vκ, Vλ, Jκ and Jλ gene segments.

[0096] 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 one or more human immunoglobulin light chain variable region gene segments, 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.

[0097] In one aspect, a genetically modified mouse is provided, wherein the mouse comprises a functionally silenced immunoglobulin light chain gene locus, and further comprises a replacement of one or more endogenous immunoglobulin light chain variable gene segments with one or more human immunoglobulin light chain variable gene segments, wherein the mouse lacks a functional endogenous ADAM6 locus, and wherein the mouse comprises an ectopic nucleotide sequence that encodes a mouse ADAM6 protein or an ortholog or homolog or fragment thereof that is functional in a male mouse.

[0098] In one embodiment, the one or more human immunoglobulin light chain variable gene segments are contiguous with the ectopic nucleotide sequence.

[0099] In one aspect, a mouse is provided that lacks a functional endogenous 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.

[00100] In one aspect, a genetically modified mouse is provided, wherein the mouse comprises a human immunoglobulin light chain variable region gene segment at an endogenous immunoglobulin heavy chain variable region gene locus, the mouse lacks an endogenous functional ADAM6 sequence at the endogenous 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.

[00101] In one aspect, a genetically modified mouse is provided, wherein the mouse comprises a human immunoglobulin light chain variable gene segment at an endogenous immunoglobulin heavy chain variable region gene locus, the mouse lacks an endogenous functional ADAM6 sequence at the endogenous immunoglobulin heavy chain variable 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.

[00102] In one embodiment, the ectopic nucleotide sequence that expresses the mouse ADAM6 protein is extra-chromosomal. 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.

[00103] In one aspect, a mouse is provided that expresses an immunoglobulin light chain sequence from a modified endogenous immunoglobulin heavy chain locus, wherein the heavy chain is derived from a human V_L gene segment, a J gene segment, and optionally a D_H gene segment, wherein the mouse comprises an ADAM6 activity that is functional in the mouse. In one embodiment, the human V_L gene segment is selected from a human $V\kappa$ and a human $V\lambda$ gene segment. In various embodiments, the J gene segment is a J_H, a $J\kappa$ or a $J\lambda$ gene segment or a combination thereof.

[00104] In one embodiment the mouse comprises a plurality of human V_L gene segments and a plurality of J gene segments. In a specific embodiment, the J gene segments are J_L gene segments.

[00105] In one aspect, a mouse is provided that expresses an immunoglobulin light chain sequence from a modified endogenous immunoglobulin heavy chain locus, wherein the heavy chain is derived from a human V_L gene segment and a J_L gene segment, wherein the mouse comprises an ADAM6 activity that is functional in the mouse.

[00106] In one embodiment, the mouse comprises a plurality of human V gene segments, a plurality of J gene segments, and optionally a plurality of D 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 a human V 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. 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 variable gene segments are incapable of encoding a heavy chain variable region 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.

[00107] In one embodiment, the plurality of human V gene segments are flanked 5' (with respect to transcriptional direction of the V gene segments) by a sequence encoding an ADAM6 activity that is functional in the mouse. In a specific embodiment, the plurality of human V gene segments include human Vκ gene segments 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 and the human Vκ2-40 gene segment is flanked 5' (with respect to transcriptional direction of the human Vκ2-40 gene segment) by a sequence encoding an ADAM6 activity that is functional in the mouse. In a specific embodiment, the sequence encoding an ADAM6 activity that is functional in the mouse is placed in the same transcription orientation with respect to the human Vκ gene segments. In a specific embodiment, the sequence encoding an ADAM6 activity that is functional in the mouse is placed in the opposite transcription orientation with respect to the human Vκ gene segments.

[00108] 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.

[00109] 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.

[00110] 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.

[00111] 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 most 3'-most V gene segment (with respect to the direction of transcription of the V gene segment) of the modified endogenous heavy chain immunoglobulin locus.

[00112] 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 V gene segment (with respect to the direction of transcription of the V gene segment) of the modified endogenous immunoglobulin heavy chain locus.

[00113] In one embodiment, the ADAM6 activity that is functional in the mouse results from expression of a nucleotide sequence located between two human V gene segments in the modified endogenous immunoglobulin heavy chain locus. In one embodiment, the two human V gene segments are a human Vκ5-2 gene segment and a Vκ4-1 gene segment.

[00114] In one embodiment, the ADAM6 activity that is functional in the mouse results from expression of a nucleotide sequence located between a human V gene segment and a human J gene segment in the modified endogenous immunoglobulin heavy chain locus. In one embodiment, the human V gene segment is a human Vκ4-1 gene segment and the human J segment is a Jκ1 gene segment.

[00115] 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.

[00116] In various embodiments, the sequence encoding an ADAM6 activity that is functional in the mouse encodes a ADAM6b protein set forth in SEQ ID NO: 2 and/or encodes a ADAM6a protein set forth in SEQ ID NO: 1.

[00117] In one embodiment, the nucleotide sequence between the two human V gene segments is placed in opposite transcription orientation with respect to the human V gene segments. In a specific embodiment, nucleotide sequence encodes, from 5' to 3' with respect to the direction of transcription of ADAM6 genes, an ADAM6a sequence followed by an ADAM6b sequence. In one embodiment, t

[00118] In one embodiment, the nucleotide sequence between the human V gene segment and the human J gene segment is placed in opposite transcription orientation with

respect to the human V and J gene segments. In a specific embodiment, the nucleotide sequence encodes, from 5' to 3' with respect to the direction of transcription of ADAM6 genes, an ADAM6a sequence followed by an ADAM6b sequence.

[00119] In one embodiment, the mouse comprises a hybrid immunoglobulin sequence, wherein the hybrid immunoglobulin sequence comprises a human immunoglobulin κ light chain sequence contiguous with a non-human ADAM6 sequence.

[00120] In one embodiment, the mouse comprises a human sequence contiguous with a mouse sequence at an endogenous immunoglobulin heavy chain locus, wherein the contiguous sequence comprises at least one human V_L gene segment, a mouse ADAM6 sequence or ortholog or homolog or functional fragment thereof, and a human J_L gene segment. In a specific embodiment, the mouse ADAM6 sequence or ortholog or homolog or functional fragment thereof is positioned immediately adjacent to the at least one human V_L gene segment. In one embodiment, the human V_L gene segment is a human $V\kappa$ gene segment. In one embodiment, the mouse ADAM6 sequence or ortholog or homolog or functional fragment thereof is positioned immediately adjacent and 3' to the at least one human V_L gene segment and immediately adjacent and 5' to the human J_L gene segment. In a specific embodiment, the human V_L gene segment is a human $V\kappa$ gene segment and the human J_L gene segment is a human $J\kappa$ gene segment.

[00121] In one embodiment, the sequence encoding the ADAM6 activity that is functional in the mouse is a mouse ADAM6 sequence or functional fragment thereof.

[00122] In one embodiment, the mouse comprises an endogenous mouse DFL16.1 gene segment (*e.g.*, in a mouse heterozygous for the modified endogenous mouse immunoglobulin heavy chain locus), or a human D_H1-1 gene segment. In one embodiment, the D gene segment of the immunoglobulin heavy chain expressed by the mouse is derived from an endogenous mouse DFL16.1 gene segment or a human D_H1-1 gene segment.

[00123] 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.

[00124] 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.

[00125] 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.

[00126] 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.

[00127] 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.

[00128] 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.

[00129] 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.

[00130] 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.

[00131] In one embodiment, the mice are incapable of expressing a functional heavy chain from each endogenous heavy chain allele, and the mice comprise a 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 immunoglobulin heavy chain locus) of a mouse immunoglobulin heavy chain constant region sequence. In a specific embodiment, the functional ADAM6 allele is at the endogenous 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. In another specific embodiment, the endogenous 90 to 100 kb intergenic V-D sequence is removed, and the ectopic ADAM6 sequence is placed between the final V and the first D gene segment.

[00132] In one aspect, a mouse is provided that comprises a modification at one or more endogenous ADAM6 alleles.

[00133] 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.

[00134] In one embodiment, the mouse is incapable of expressing a functional ADAM6 protein from each endogenous ADAM6 allele, and the mice comprise an ectopic ADAM6 sequence.

[00135] In one embodiment, the mouse is incapable of expressing a functional ADAM6 protein from each endogenous ADAM6 allele, and the mouse comprises 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 J 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 human Vκ gene segment and a first human Jκ 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 a human Vκ gene segment, wherein the human Vκ gene segment is selected from a human Vκ4-1 or Vκ2-40 gene segment.

[00136] In one embodiment, the mouse is capable of expressing a functional ADAM6 protein from one or more endogenous ADAM6 alleles and the modification includes an insertion of a human sequence that encodes an immunoglobulin variable domain. In one embodiment, the human sequence comprises unrearranged immunoglobulin gene segments. In a specific embodiment, the human sequence comprises a V gene segment and a J gene segment. In another specific embodiment, the human sequence comprises a V gene segment, a J gene segment, and a D gene segment.

[00137] 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.

[00138] 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. In various embodiments, the majority of the B cells further comprise one or more human V_L and J_L gene segments upstream of a mouse immunoglobulin heavy chain constant region. In one embodiment, the human V_L and J_L gene segments are Vκ and Jκ gene segments.

[00139] 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.

[00140] In one aspect, a method is provided for making an infertile male mouse, 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 mouse.

[00141] In one aspect, a method is provided for making a mouse with a genetic modification of interest, wherein the mouse 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 mouse. In various embodiments, the genome is from an ES cell or used in a nuclear transfer experiment.

[00142] 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.

[00143] 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.

[00144] 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 or more non-mouse immunoglobulin variable region sequences, and wherein the mouse comprises a nucleic acid sequence encoding a mouse ADAM6 protein. In one 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 light chain sequences. In one embodiment, the D_H, J_H, and all V_H sequences of the endogenous immunoglobulin heavy chain locus are replaced by a plurality of non-mouse immunoglobulin V_L gene segments, one or more J_L gene segment, and optionally one or more D gene segment sequences. The non-mouse immunoglobulin sequences can be unrearranged. In one embodiment, the non-mouse immunoglobulin sequences comprise complete unrearranged V_L and J_L regions of the non-mouse species. In one embodiment, the non-mouse immunoglobulin sequences are capable of forming a complete variable region, *i.e.*, a rearranged variable region containing V_L and J_L gene segments joined together to form an sequence that encodes a light chain variable region, of the non-mouse species, operably linked to one or more endogenous constant regions. The non-mouse species can be Homo sapiens and the non-mouse immunoglobulin sequences can be human sequences.

[00145] In one aspect, a genetically modified mouse comprising a nucleotide sequence encoding an ADAM6 protein or functional fragment thereof that is contiguous with a human immunoglobulin light chain variable gene segment is provided.

[00146] In one embodiment, the mouse lacks an endogenous unmodified ADAM6 gene sequence. In one embodiment, the mouse lacks a functional endogenous ADAM6 gene sequence.

[00147] In one embodiment, the human immunoglobulin light chain variable gene segment is an immunoglobulin κ light chain variable gene segment. In one embodiment, the human immunoglobulin light chain variable gene segment is an immunoglobulin λ light chain variable gene segment. In one embodiment, the human immunoglobulin light chain variable gene segment is operably linked to an immunoglobulin heavy chain constant gene sequence.

[00148] In one embodiment, the immunoglobulin heavy chain constant gene sequence is a mouse or rat or human heavy chain gene sequence. In one embodiment, the heavy chain constant gene sequence comprises a C_H1 and/or a hinge region.

[00149] In one embodiment, the mouse comprises deletion of, or replacement of, one or more endogenous immunoglobulin heavy chain gene sequences.

[00150] In one embodiment, the mouse further comprises an unrearranged human Vκ or unrearranged human Vλ gene segment operably linked to a human or mouse or rat light chain constant region sequence. In one embodiment, the mouse comprises a plurality of unrearranged human Vκ gene segments (*e.g.,* two or more human Vκ segments and one or more human Jκ segments) or a plurality of unrearranged human Vλ gene segments (*e.g.*, two or more human Vλ segments and one or more human Jλ segments). In one embodiment, the unrearranged human Vκ or unrearranged human Vλ gene segments are operably linked to a constant region sequence at an endogenous immunoglobulin light chain locus.

[00151] In one embodiment, the mouse further comprises a modification that renders an endogenous κ light chain locus and/or an endogenous λ light chain locus nonfunctional. In one embodiment, the mouse comprises a knockout or a deletion of an endogenous mouse κ and/or an endogenous mouse λ light chain locus.

[00152] In one aspect, a method for maintaining a mouse strain is provided, wherein the mouse strain comprises a replacement of a mouse immunoglobulin heavy chain sequence with one or more human immunoglobulin light chain sequences. In one embodiment, the one or more human immunoglobulin light chain sequences are human immunoglobulin Vκ and/or Jκ gene segments.

[00153] In one embodiment, the mouse strain comprises a deletion of one or more mouse V_H, D_H, and/or J_H gene segments. In one embodiment, the mouse further comprises one or more human V_L gene segments and one or more human J_L gene segments. In one embodiment, the mouse comprises at least 6, at least 16, at least 30, or at least 40 human Vκ gene segments and at least five Jκ gene segments. In a specific embodiment, the human light chain gene segments are operably linked to a constant region gene. In one embodiment, the constant region gene is a mouse constant region gene. In one embodiment, the constant region gene comprises a mouse 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.

[00154] In one embodiment, the method comprises generating a male mouse heterozygous for the replacement of the mouse immunoglobulin heavy chain sequence, and breeding the heterozygous male mouse with a wild-type female mouse or a female mouse that is homozygous or heterozygous for the human heavy chain sequence. In one embodiment, the method comprises maintaining the strain by repeatedly breeding heterozygous males with females that are wild type or homozygous or heterozygous for the human heavy chain sequence.

[00155] In one embodiment, the method comprises obtaining cells from male or female

mice 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 animals using host cells and/or gestating the cells and/or nuclei in surrogate mothers.

[00156] In one embodiment, only male mice that are heterozygous for the replacement at the heavy chain locus are bred to female mice. In a specific embodiment, the female mice are homozygous, heterozygous, or wild type with respect to a replaced heavy chain locus.

[00157] In one embodiment, the mouse further comprises 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.

[00158] In one embodiment, the mouse 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 VJ) 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.

[00159] In one aspect, a fertile mouse is provided that comprises a modification of an endogenous ADAM6 gene, wherein the mouse comprises an ectopic sequence that confers ADAM6 function to the mouse, and wherein the mouse comprises in its germline an unrearranged human immunoglobulin light chain gene segment operably linked to a nucleic acid sequence encoding an immunoglobulin heavy chain sequence.

[00160] In one aspect, a fertile mouse is provided that comprises a modification of an endogenous ADAM6 locus, wherein the modification renders the ADAM6 locus nonfunctional, and wherein the mouse expresses an immunoglobulin binding protein comprising a human immunoglobulin light chain variable domain contiguous with a heavy chain constant sequence.

[00161] In one embodiment, the immunoglobulin binding protein further comprises a cognate human immunoglobulin light chain variable domain fused with a light chain constant sequence.

[00162] In one embodiment, the heavy chain constant sequence and the light chain constant sequence are non-human.

[00163] In one aspect, a mouse is provided, comprising an immunoglobulin heavy chain locus comprising a replacement of one or more immunoglobulin heavy chain variable region (V_H) gene segments, heavy chain diversity (D_H) gene segments, and heavy chain joining (J_H) gene segments at an endogenous immunoglobulin heavy chain locus with one or more light chain variable region (V_L) gene segments and one or more light chain joining region (J_L) gene segments, wherein the mouse is capable of expressing an ADAM6 protein.

[00164] In one aspect, a mouse is provided, comprising an immunoglobulin heavy chain locus that comprises a replacement of all or substantially all V_H, D_H, and J_H gene segments with one or more V_L gene segments and one or more J_L gene segments to form a V_L gene segment sequence at an endogenous heavy chain locus (VL_H locus), wherein the VL_H locus is capable of recombining with an endogenous C_H gene to form a rearranged gene that is derived from a V_L gene segment, a J_L gene segment, and an endogenous C_H gene.

[00165] In one embodiment, the V_L segments are human V_L. In one embodiment, the J_L segments are human J_L. In a specific embodiment, the V_L and J_L segments are human V_L and human J_L segments.

[00166] In one embodiment, all or substantially all V_H, D_H, and J_H gene segments are replaced with at least six human Vκ gene segments and at least one Jκ gene segment. In one embodiment, all or substantially all V_H, D_H, and J_H 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 V_H, D_H, and J_H 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 V_H, D_H, and J_H 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.

[00167] In one embodiment, the V_L segments are human Vκ segments. In one embodiment, the human Vκ segments comprise 4-1, 5-2, 7-3, 2-4, 1-5, and 1-6. In one embodiment, the human Vκ segments comprise 3-7, 1-8, 1-9, 2-10, 3-11, 1-12, 1-13, 2-14, 3-15, and 1-16. In one embodiment, the human Vκ segments comprise 1-17, 2-18, 2-19, 3-20, 6-21, 1-22, 1-23, 2-24, 3-25, 2-26, 1-27, 2-28, 2-29, and 2-30. In one embodiment, the

human Vκ segments comprise 3-31, 1-32, 1-33, 3-34, 1-35, 2-36, 1-37, 2-38, 1-39, and 2-40.

[00168] In one embodiment, the V_L segments are human Vκ segments and comprise 4-1, 5-2, 7-3, 2-4, 1-5, 1-6, 3-7, 1-8, 1-9, 2-10, 3-11, 1-12, 1-13, 2-14, 3-15, and 1-16. In one embodiment, the Vκ segments further comprise 1-17, 2-18, 2-19, 3-20, 6-21, 1-22, 1-23, 2-24, 3-25, 2-26, 1-27, 2-28, 2-29, and 2-30. In one embodiment, the Vκ segments further comprise 3-31, 1-32, 1-33, 3-34, 1-35, 2-36, 1-37, 2-38, 1-39, and 2-40.

[00169] In one embodiment, the V_L segments are human Vλ segments and comprise a fragment of cluster A of the human λ light chain locus. In a specific embodiment, the fragment of cluster A of the human λ light chain locus extends from hVλ3-27 through hVλ3-1.

[00170] In one embodiment, the V_L segments comprise a fragment of cluster B of the human λ light chain locus. In a specific embodiment, the fragment of cluster B of the human λ light chain locus extends from hVλ5-52 through hVλ1-40.

[00171] In one embodiment, the V_L segments comprise a human λ light chain variable region sequence that comprises a genomic fragment of cluster A and a genomic fragment of cluster B. In a one embodiment, the human λ light chain variable region sequence comprises at least one gene segment of cluster A and at least one gene segment of cluster B.

[00172] In one embodiment, the V_L segments comprise at least one gene segment of cluster B and at least one gene segment of cluster C.

[00173] In one embodiment, the V_L segments comprise hVλ 3-1, 4-3, 2-8, 3-9, 3-10, 2-11, and 3-12. In a specific embodiment, the V_L segments comprise a contiguous sequence of the human λ light chain locus that spans from Vλ3-12 to Vλ3-1. In one embodiment, the contiguous sequence comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 hVλs. In a specific embodiment, the hVλs include 3-1, 4-3, 2-8, 3-9, 3-10, 2-11, and 3-12. In a specific embodiment, the hVλs comprises a contiguous sequence of the human λ locus that spans from Vλ3-12 to Vλ3-1.

[00174] In one embodiment, the hVλs comprises 13 to 28 or more hVλs. In a specific embodiment, the hVλs include 2-14, 3-16, 2-18, 3-19, 3-21, 3-22, 2-23, 3-25, and 3-27. In a specific embodiment, the hVλs comprise a contiguous sequence of the human λ locus that spans from Vλ3-27 to Vλ3-1.

[00175] In one embodiment, the V_L segments comprise 29 to 40 hVλs. In a specific embodiment, the V_L segments comprise a contiguous sequence of the human λ locus that

spans from Vλ3-29 to Vλ3-1, and a contiguous sequence of the human λ locus that spans from Vλ5-52 to Vλ1-40. In a specific embodiment, all or substantially all sequence between hVλ1-40 and hVλ3-29 in the genetically modified mouse consists essentially of a human λ sequence of approximately 959 bp found in nature (*e.g.,* in the human population) downstream of the hVλ1-40 gene segment (downstream of the 3' untranslated portion), a restriction enzyme site (*e.g.,* PI-SceI), followed by a human λ sequence of approximately 3,431 bp upstream of the hVλ3-29 gene segment found in nature.

[00176] In one embodiment, the Jκ is human and is selected from the group consisting of Jκ1, Jκ2, Jκ3, Jκ4, Jκ5, and a combination thereof. In a specific embodiment, the Jκ comprises Jκ1 through Jκ5.

[00177] In one embodiment, the V_L segments are human Vλ segments, and the Jκ gene segment comprises an RSS having a 12-mer spacer, wherein the RSS is juxtaposed at the upstream end of the Jκ gene segment. In one embodiment, the V_L gene segments are human Vλ and the VL_H locus comprises two or more Jκ gene segments, each comprising an RSS having a 12-mer spacer wherein the RSS is juxtaposed at the upstream end of each Jκ gene segment.

[00178] In a specific embodiment, the V_L segments comprise contiguous human κ gene segments spanning the human κ locus from Vκ4-1 through Vκ2-40, and the J_L segments comprise contiguous gene segments spanning the human κ locus from Jκ1 through Jκ5.

[00179] In one embodiment, where the V_L segments are Vλ segments and no D_H segment is present between the V_L segments and J segments, the V_L segments are flanked downstream (*i.e.*, juxtaposed on the downstream side) with 23-mer RSS, and Jκ segments if present or Jλ segments if present are flanked upstream (*i.e.,* juxtaposed on the upstream side) with 12-mer RSS.

[00180] In one embodiment, where the V gene segments are Vκ gene segments and no D_H gene segment is present between the V gene segments and J gene segments, the Vκ gene segments are each juxtaposed on the downstream side with a 12-mer RSS, and Jκ segments if present or Jλ segments if present are each juxtaposed on the upstream side with a 23-mer RSS.

[00181] In one embodiment, the mouse comprises a rearranged gene that is derived from a V_L gene segment, a J_L gene segment, and an endogenous C_H gene. In one embodiment, the rearranged gene is somatically mutated. In one embodiment, the rearranged gene comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more N additions. In one embodiment, the N additions and/or the somatic mutations observed in the rearranged gene derived from the V_L

segment and the J_L segment are 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, or at least 5-fold more than the number of N additions and/or somatic mutations observed in a rearranged light chain variable domain (derived from the same V_L gene segment and the same J_L gene segment) that is rearranged at an endogenous light chain locus. In one embodiment, the rearranged gene is in a B cell that specifically binds an antigen of interest, wherein the B cell binds the antigen of interest with a K_D in the low nanomolar range or lower (*e.g.*, a K_D of 10 nanomolar or lower). In a specific embodiment, the V_L segment, the J_L segment, or both, are human gene segments. In a specific embodiment, the V_L and J_L segments are human κ gene segments. In one embodiment, the mouse C_H gene is selected from IgM, IgD, IgG, IgA and IgE. In a specific embodiment, the mouse IgG is selected from IgG1, IgG2A, IgG2B, IgG2C and IgG3. In another specific embodiment, the mouse IgG is IgG1.

[00182] In one embodiment, the mouse comprises a B cell, wherein the B cell expresses from a locus on a chromosome of the B cell a binding protein consisting essentially of four polypeptide chains, wherein the four polypeptide chains consist essentially of (a) two identical polypeptides that comprise an endogenous C_H region fused with a V_L; and, (b) two identical polypeptides that comprise an endogenous C_L region fused with a V_L region that is cognate with respect to the V_L region that is fused with the mouse C_H region, and, in one embodiment, is a human *(e.g.*, a human κ) V_L region. In one embodiment, the V_L region fused to the endogenous C_H region is a human V_L region. In a specific embodiment, the human V_L region fused with the mouse C_H region is a Vκ region. In a specific embodiment, the human V_L region fused with the mouse C_H region is identical to a V region encoded by a rearranged human germline light chain nucleotide sequence. In a specific embodiment, the human V_L region fused to the mouse C_H region comprises two, three, four, five, six, or more somatic hypermutations. In one embodiment, the human V_L region fused to the mouse C_H region is encoded by a rearranged gene that comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more N additions.

[00183] In one embodiment, at least 50% of all IgG molecules expressed by the mouse comprise a polypeptide that comprises an IgG isotype C_H region and a V_L region, wherein the length of said polypeptide is no longer than 535, 530, 525, 520, or 515 amino acids. In one embodiment, at least 75% of all IgG molecules comprise the polypeptide recited in this paragraph. In one embodiment, at least 80%, 85%, 90%, or 95% of all IgG molecules comprise the polypeptide recited in this paragraph. In a specific embodiment, all IgG molecules expressed by the mouse comprise a polypeptide that is no longer than the length of the polypeptide recited in this paragraph.

[00184] In one embodiment, the mouse expresses a binding protein comprising a first polypeptide that comprises an endogenous C_H region fused with a variable domain encoded by a rearranged human V gene segment and a J gene segment but not a D_H gene segment, and a second polypeptide that comprises an endogenous C_L region fused with a V domain encoded by a rearranged human V gene segment and a J gene segment but not a D_H gene segment, and the binding protein specifically binds an antigen with an affinity in the micromolar, nanomolar, or picomolar range. In one embodiment, the J segment is a human J segment (*e.g.*, a human κ gene segment). In one embodiment, the human V segment is a human Vκ segment. In one embodiment, the variable domain that is fused with the endogenous C_H region comprises a greater number of somatic hypermutations than the variable region that is fused with the endogenous C_L region; in a specific embodiment, the variable region fused with the endogenous C_H region comprises about 1.5, 2-, 3-, 4-, or 5-fold or more somatic hypermutations than the V region fused to the endogenous C_L region; in a specific embodiment, the V region fused with the mouse C_H region comprises at least 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 or more somatic hypermutations than the V region fused with the mouse C_L region. In one embodiment, the V region fused with the mouse C_H region is encoded by a rearranged gene that comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more N additions.

[00185] In one embodiment, the mouse expresses a binding protein comprising a first light chain variable domain (V_L1) fused with an immunoglobulin heavy chain constant region sequence and a second light chain variable domain (V_L2) fused with an immunoglobulin light chain constant region, wherein V_L1 comprises a number of somatic hypermutations that is about 1.5- to about 5-fold higher or more than the number of somatic hypermutations present in V_L2. In one embodiment, the number of somatic hypermutations in V_L1 is about 2- to about 4-fold higher than in V_L2. In one embodiment, the number of somatic hypermutations in V_L1 is about 2- to about 3-fold higher than in V_L2. In one embodiment, V_L1 is encoded by a sequence that comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more N additions.

[00186] In one aspect, a genetically modified mouse is provided that expresses an immunoglobulin that consists essentially of the following polypeptides: a first two identical polypeptides that each consists essentially of a C_H region fused with a variable domain that is derived from gene segments that consist essentially of a V_L gene segment and a J_L gene segment, and a second two identical polypeptides that each consists essentially of a C_L region fused with a variable domain that is derived from gene segments that consist essentially of a V_L segment and a J_L segment.

[00187] In a specific embodiment, the two identical polypeptides that have the C_H region have a mouse C_H region.

[00188] In a specific embodiment, the two identical polypeptides that have the C_L region have a mouse C_L region.

[00189] In one embodiment, the variable domain fused with the C_L region is a variable domain that is cognate with the variable domain fused to the C_H region.

[00190] In one embodiment, the variable domain that is fused with the endogenous C_H region comprises a greater number of somatic hypermutations than the variable domain that is fused with the endogenous C_L region; in a specific embodiment, the variable domain fused with the endogenous C_H region comprises about 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, or 5-fold or more somatic hypermutations than the variable domain fused to the endogenous C_L region. In one embodiment, the variable domain fused with the endogenous C_L region is encoded by a gene that comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more N additions.

[00191] In one embodiment, one or more of the V segments and the J segments are human gene segments. In a specific embodiment, both the V segments and the J segments are human κ gene segments. In another specific embodiment, both of the V segments and the J segments are human λ gene segments. In one embodiment, the V segments and the J segments are independently selected from human κ and human λ gene segments. In a specific embodiment, the V segments are Vκ segments and the J segments are Jλ segments. In another specific embodiment, the V segments are Vλ segments and the J segments are Jκ segments.

[00192] In one embodiment, one or more of the variable domains fused with the C_L region and the variable domains fused with the C_H region are human variable domains. In a specific embodiment, the human variable domains are human Vκ domains. In another specific embodiment, the human variable domains are Vλ domains. In one embodiment, the human domains are independently selected from human Vκ and human Vλ domains. In a specific embodiment, the human variable domain fused with the C_L region is a human Vλ domain and the human variable domain fused with the C_H region is a human Vκ domain. In another embodiment, the human variable domain fused with the C_L region is a human Vκ domain and the human variable domain fused with the C_H is a human Vλ domain.

[00193] In one embodiment, the V_L gene segment of the first two identical polypeptides is selected from a human Vλ segment and a human Vκ segment. In one embodiment, the V_L segment of the second two identical polypeptides is selected from a human Vλ segment and a human Vκ segment. In a specific embodiment, the V_L segment of the first two identical

polypeptides is a human Vκ segment and the V_L segment of the second two identical polypeptides is selected from a human Vκ segment and a human Vλ segment. In a specific embodiment, the V_L segment of the first two identical polypeptides is a human Vλ segment and the V_L segment of the second two identical polypeptides is selected from a human Vλ segment and a human Vκ segment. In a specific embodiment, the human V_L segment of the first two identical polypeptides is a human Vκ segment, and the human V_L segment of the second two identical polypeptides is a human Vκ segment.

[00194] In one embodiment, the IgG of the mouse comprises a binding protein made in response to an antigen, wherein the binding protein comprises a polypeptide that consists essentially of a variable domain and a C_H region, wherein the variable domain is encoded by a nucleotide sequence that consists essentially of a rearranged V_L segment and a rearranged J segment, and wherein the binding protein specifically binds an epitope of the antigen with a K_D in the micromolar, nanomolar, or picomolar range.

[00195] In one aspect, a mouse is provided, wherein all or substantially all of the IgG made by the mouse in response to an antigen comprises a heavy chain that comprises a variable domain, wherein the variable domain is encoded by a rearranged gene derived from gene segments that consist essentially of a V gene segment and a J gene segment. In one embodiment, the rearranged gene comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more N additions.

[00196] In one embodiment, the V segment is a V segment of a light chain. In one embodiment, the light chain is selected from a κ light chain and a λ light chain. In a specific embodiment, the light chain is a κ light chain. In a specific embodiment, the V segment is a human V segment. In a specific embodiment, the V segment is a human Vκ segment and the J segment is a human Jκ segment.

[00197] In one embodiment, the J segment is a J segment of a light chain. In one embodiment, the light chain is selected from a κ light chain and a λ light chain. In a specific embodiment, the light chain is a κ light chain. In a specific embodiment, the J segment is a human J segment. In another embodiment, the J segment is a J segment of a heavy chain (*i.e.*, a J_H). In a specific embodiment, the heavy chain is of mouse origin. In another specific embodiment, the heavy chain is of human origin.

[00198] In one embodiment, the variable domain of the heavy chain that is made from no more than a V segment and a J segment is a somatically mutated variable domain.

[00199] In one embodiment, the variable domain of the heavy chain that is made from no more than a V segment and a J segment is fused to a mouse C_H region.

[00200] In a specific embodiment, all or substantially all of the IgG made by the mouse in response to an antigen comprises a variable domain that is derived from no more than one human V segment and no more than one human J segment, and the variable domain is fused to a mouse IgG constant region, and the IgG further comprises a light chain that comprises a human V_L domain fused with a mouse C_L region. In a specific embodiment, the V_L domain fused with the mouse C_L region is derived from a human $V\kappa$ segment and a human $J\kappa$ segment. In a specific embodiment, the V_L domain fused with the mouse C_L region is derived from a human $V\lambda$ segment and a human $J\lambda$ segment.

[00201] In one aspect, a mouse is provided that makes an IgG comprising a first CDR3 on a polypeptide comprising a C_H region and a second CDR3 on a polypeptide comprising a C_L region, wherein both the first CDR3 and the second CDR3 are each independently derived from no more than two gene segments, wherein the two gene segments consist essentially of a V_L gene segment and a J_L gene segment. In one embodiment, the CDR3 on the polypeptide comprising the C_H region comprises a sequence that is derived from a CDR3 nucleotide sequence that comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more N additions.

[00202] In one embodiment, the V_L segment and the J_L segment are human gene segments. In one embodiment, the V_L segment and the J_L segment are κ gene segments. In one embodiment, the V_L segment and the J_L segment are λ gene segments.

[00203] In one aspect, a mouse is provided that makes an IgG comprising a first CDR3 on a first polypeptide comprising a C_H region and a second CDR3 on a second polypeptide comprising a C_L region, wherein both the first CDR3 and the second CDR3 each comprise a sequence of amino acids wherein more than 75% of the amino acids are derived from a V gene segment. In one embodiment, the CDR3 on the polypeptide comprising the C_H region comprises a sequence that is derived from a CDR3 nucleotide sequence that comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more N additions.

[00204] In one embodiment, more than 80%, more than 90%, or more than 95% of the amino acids of the first CDR3, and more than 80%, more than 90%, or more than 95% of the amino acids of the second CDR3, are derived from a light chain V segment.

[00205] In one embodiment, no more than two amino acids of the first CDR3 are derived from a gene segment other than a light chain V segment. In one embodiment, no more than two amino acids of the second CDR3 are derived from a gene segment other than a light chain V segment. In a specific embodiment, no more than two amino acids of the first CDR3 and no more than two amino acids of the second CDR3 are derived from a gene segment other than a light chain V segment. In one embodiment, no CDR3 of the IgG comprises an amino acid sequence derived from a D gene segment. In one embodiment, the CDR3 of the first polypeptide does not comprise a sequence derived from a D segment.

[00206] In one embodiment, the V segment is a human V gene segment. In a specific embodiment, the V segment is a human V_κ gene segment.

[00207] In one embodiment, the first and/or the second CDR3 have at least one, two, three, four, five, or six somatic hypermutations. In one embodiment, the first CDR3 is encoded by a nucleic acid sequence that comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more N additions.

[00208] In one embodiment, the first CDR3 consists essentially of amino acids derived from a human light chain V gene segment and a human light chain J gene segment, and the second CDR3 consists essentially of amino acids derived from a human light chain V gene segment and a human light chain J gene segment. In one embodiment, the first CDR3 is derived from a nucleic acid sequence that comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more N additions. In one embodiment, the first CDR3 is derived from no more than two gene segments, wherein the no more than two gene segments are a human V_κ gene segment and a human J_κ gene segment; and the second CDR3 is derived from no more than two gene segments, wherein the no more than two gene segments are a human V_κ gene segment and a J gene segment selected from a human J_κ segment, a human J_λ segment, and a human J_H segment. In one embodiment, the first CDR3 is derived from no more than two gene segments, wherein the no more than two gene segments are a human V_λ segment and a J segment selected from a human J_κ segment, a human J_λ segment, and a human J_H segment.

[00209] In one aspect, a mouse is provided that makes an IgG that does not contain an amino acid sequence derived from a D_H gene segment, wherein the IgG comprises a first polypeptide having a first V_L domain fused with a mouse C_L region and a second polypeptide having a second V_L domain fused with a mouse C_H region, wherein the first V_L domain and the second V_L domain are not identical. In one embodiment, the first and second V_L domains are derived from different V segments. In another embodiment, the first and second V_L domains are derived from different J segments. In one embodiment, the first and second V_L domains are derived from identical V and J segments, wherein the second V_L domain comprises a higher number of somatic hypermutations as compared to the first V_L domain.

[00210] In one embodiment, the first and the second V_L domains are independently selected from human and mouse V_L domains. In one embodiment, the first and second V_L domains are independently selected from V_κ and V_λ domains. In a specific embodiment, the first V_L domain is selected from a V_κ domain and a V_λ domain, and the second V_L

domain is a Vκ domain. In another specific embodiment, the Vκ domain is a human Vκ domain.

[00211] In one aspect, a mouse is provided, wherein all or substantially all of the IgG made by the mouse consists essentially of a light chain having a first human V_L domain fused with a mouse C_L domain, and a heavy chain having a second human V_L domain fused with a mouse C_H domain.

[00212] In one embodiment, the human V_L domain fused with the mouse C_H domain is a human Vκ domain.

[00213] In one embodiment, the first and the second human V_L domains are not identical.

[00214] In one aspect, a mouse is provided, wherein at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or about 100% of the immunoglobulin G made by the mouse consists essentially of a dimer of (a) a first polypeptide that consists essentially of an immunoglobulin V_L domain and an immunoglobulin C_L region; and, (b) a second polypeptide of no more than 535 amino acids in length, wherein the second polypeptide consists essentially of a C_H region and a V domain that lacks a sequence derived from a D_H gene segment.

[00215] In one embodiment, the second polypeptide is about 435-535 amino acids in length. In a specific embodiment, the second polypeptide is about 435-530 amino acids in length. In a specific embodiment, the second polypeptide is about 435-525 amino acids in length. In a specific embodiment, the second polypeptide is about 435-520 amino acids in length. In a specific embodiment, the second polypeptide is about 435-515 amino acids in length.

[00216] In one embodiment, in about 90% or more of the IgG made by the mouse the second polypeptide is no more than about 535 amino acids in length.

[00217] In one embodiment, in about 50% or more of the IgG made by the mouse the second polypeptide is no more than about 535 amino acids in length. In one embodiment, in about 50% or more of the immunoglobulin G made by the mouse the second polypeptide is no more than about 530, 525, 520, 515, 510, 505, 500, 495, 490, 485, 480, 475, 470, 465, 460, 455, or 450 amino acids in length. In one embodiment, about 60%, 70%, 80%, 90%, or 95 % or more of the IgG made by the mouse is of the recited length. In a specific embodiment, all or substantially all of the IgG made by the mouse is of the recited length.

[00218] In one embodiment, the V domain of the second polypeptide is a V_L domain. In a specific embodiment, the V domain of the second polypeptide is selected from a Vκ and a Vλ domain. In a specific embodiment, the V domain of the second polypeptide is a human Vκ or Vλ domain.

[00219] In one aspect, a mouse is provided that expresses from a nucleotide sequence in its germline a polypeptide that comprises a light chain variable sequence (*e.g.*, a V and/or J sequence), a D_H sequence, and a heavy chain constant region.

[00220] In one embodiment, the mouse expresses the polypeptide from an endogenous heavy chain locus that comprises a replacement of all or substantially all functional endogenous heavy chain variable locus gene segments with a plurality of human gene segments at the endogenous heavy chain locus.

[00221] In one embodiment, the polypeptide comprises a V_L sequence derived from a Vλ or a Vκ gene segment, the polypeptide comprises a CDR3 derived from a D_H gene segment, and the polypeptide comprises a sequence derived from a J_H or Jλ or Jκ gene segment.

[00222] In one embodiment, the mouse comprises an endogenous heavy chain immunoglobulin locus comprising a replacement of all functional V_H gene segments with one or more human light chain Vλ gene segments wherein the one or more human Vλ segments each have juxtaposed on the downstream side a 23-mer spaced recombination signal sequence (RSS), wherein the Vλ segments are operably linked to a human or mouse D_H segment that has juxtaposed upstream and downstream a 12-mer spaced RSS; the D_H gene segment is operably linked with a J segment juxtaposed upstream with a 23-mer spaced RSS that is suitable for recombining with the 12-mer spaced RSS juxtaposing the D_H gene segment; wherein the V, D_H, and J segments are operably linked to a nucleic acid sequence encoding a heavy chain constant region.

[00223] In one embodiment, the mouse comprises an endogenous heavy chain immunoglobulin locus comprising a replacement of all functional V_H gene segments with one or more human Vκ gene segments each juxtaposed on the downstream side with a 12-mer spaced recombination signal sequence (RSS), wherein the V segments are operably linked to a human or mouse D_H segment that is juxtaposed both upstream and downstream with a 23-mer spaced RSS; the D_H segment is operably linked with a J segment juxtaposed on the upstream side with a 12-mer spaced RSS that is suitable for recombining with the 23-mer spaced RSS juxtaposing the D_H segment; wherein the V, D_H, and gene segments are operably linked to a nucleic acid sequence encoding a heavy chain constant region.

[00224] In one embodiment, the heavy chain constant region is an endogenous heavy chain constant region. In one embodiment, the nucleic acid sequence encodes a sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, one or more of the C_H1, hinge, C_H2, and C_H3 are human.

[00225] In one embodiment, the mouse comprises an endogenous heavy chain immunoglobulin locus comprising a replacement of all functional V_H gene segments with a

plurality of human Vλ or Vκ gene segments each juxtaposed downstream with 23-mer spaced RSS, a plurality of human D_H segments juxtaposed both upstream and downstream by a 12-mer spaced RSS, a plurality of human J segments (J_H or Jλ or Jκ) juxtaposed both upstream and downstream with a 23-mer spaced RSS, wherein the locus comprises an endogenous constant region sequence selected from C_H1, hinge, C_H2, C_H3, and a combination thereof. In a specific embodiment, the mouse comprises all or substantially all functional human Vλ or Vκ segments, all or substantially all functional human D_H segments, and all or substantially all J_H or Jλ or Jκ segments.

[00226] In one embodiment, the mouse expresses an antigen-binding protein comprising (a) a polypeptide that comprises a human light chain sequence linked to a heavy chain constant sequence comprising a mouse sequence; and (b) a polypeptide that comprises a human light chain variable region linked to a human or mouse light chain constant sequence. In a specific embodiment, the light chain sequence is a human light chain sequence, and upon exposure to a protease that is capable of cleaving an antibody into an Fc and a Fab, a fully human Fab is formed that comprises at least four light chain CDRs, wherein the at least four light chain CDRs are selected from λ sequences, κ sequences, and a combination thereof. In one embodiment, the Fab comprises at least five light chain CDRs. In one embodiment, the Fab comprises six light chain CDRs. In one embodiment, at least one CDR of the Fab comprises a sequence derived from a Vλ segment or a Vκ segment, and the at least one CDR further comprises a sequence derived from a D segment. In one embodiment, the at least one CDR is a CDR3 and the CDR is derived from a human Vκ segment, a human D segment, and a human Jκ segment.

[00227] In one embodiment, the polypeptide of comprises a variable region derived from a human Vλ or Vκ gene segment, a human D_H gene segment, and a human J_H or Jλ or Jκ gene segment. In a specific embodiment, the heavy chain constant sequence is derived from a human C_H1 and a mouse C_H2 and a mouse C_H3 sequence.

[00228] In one aspect, a mouse is provided that comprises in its germline an unrearranged human Vκ or Vλ gene segment operably linked to a human J gene segment and a heavy chain constant region sequence, wherein the mouse expresses a V_L binding protein that comprises a human Vκ domain fused with a heavy chain constant region and a human V_L domain fused with a light chain constant domain. In one embodiment, the human V_L domain comprises a rearranged human V_L gene segment selected from a human Vκ and a human Vλ gene segment. In a specific embodiment, the human V_L domain further comprises a rearranged human J_L gene segment selected from a human Jκ and a human Jλ gene segment.

[00229] In one aspect, a mouse is provided that expresses an immunoglobulin protein from a modified endogenous heavy chain locus in its germline, wherein the modified endogenous heavy chain locus lacks a functional mouse heavy chain V gene segment and the locus comprises unrearranged light chain V gene segments and unrearranged J gene segments, wherein the unrearranged light chain V gene segments and unrearranged J gene segments are operably linked with a heavy chain constant region sequence; wherein the immunoglobulin protein consists essentially of a first polypeptide and a second polypeptide, wherein the first polypeptide comprises an immunoglobulin light chain sequence and an immunoglobulin heavy chain constant sequence, and the second polypeptide comprises an immunoglobulin light chain variable domain and a light chain constant region.

[00230] In one aspect, a mouse is provided that expresses an immunoglobulin protein, wherein the immunoglobulin protein lacks a heavy chain immunoglobulin variable domain, and the immunoglobulin protein comprises a first variable domain derived from a light chain gene, and a second variable domain derived from a light chain gene, wherein the first variable domain and the second variable domain are cognate with respect to one another, wherein the first and the second light chain variable domains are not identical, and wherein the first and the second light chain variable domains associate and when associated specifically bind an antigen of interest.

[00231] In one aspect, a mouse is provided that expresses from unrearranged gene segments in its germline an immunoglobulin protein comprising variable regions that are wholly derived from gene segments that consist essentially of unrearranged human gene segments, wherein the immunoglobulin protein comprises an immunoglobulin light chain constant sequence and an immunoglobulin heavy chain constant sequence selected from the group consisting of a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof.

[00232] In one aspect, a mouse is provided that expresses from unrearranged gene segments in its germline an immunoglobulin protein comprising variable regions, wherein all CDR3s of all variable regions are generated entirely from light chain V and J gene segments, and optionally one or more somatic hypermutations, *e.g.*, one or more N additions.

[00233] In one aspect, a mouse is provided that expresses a somatically mutated immunoglobulin protein derived from unrearranged human immunoglobulin light chain variable region gene segments in the germline of the mouse, wherein the immunoglobulin protein lacks a CDR that comprises a sequence derived from a D gene segment, wherein the immunoglobulin protein comprises a first CDR3 on a light chain variable domain fused with a light chain constant region, comprises a second CDR3 on a light chain variable domain fused with a heavy chain constant region, and wherein the second CDR3 is derived

from a rearranged light chain variable region sequence that comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more N additions.

[00234] In one aspect, a mouse as described herein is provided, wherein the mouse comprises a functionally silenced light chain locus selected from a λ locus, a κ locus, and a combination thereof. In one embodiment, the mouse comprises a deletion of a λ and/or a κ locus, in whole or in part, such that the λ and/or κ locus is nonfunctional.

[00235] In one aspect, a mouse embryo is provided, comprising a cell that comprises a modified immunoglobulin locus as described herein. In one embodiment, the mouse is a chimera and at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the cells of the embryo comprise a modified immunoglobulin locus as described herein. In one embodiment, at least 96%. 97%, 98%, 99%, or 99.8% of the cells of the embryo comprise a modified immunoglobulin locus as described herein. In one embodiment, the embryo comprises a host cell and a cell derived from a donor ES cell, wherein the cell derived from the donor ES cell comprises a modified immunoglobulin locus as described herein. In one embodiment, the embryo is a 2-, 4-, 8, 16-, 32, or 64-cell stage host embryo, or a blastocyst, and further comprises a donor ES cell comprising a modified immunoglobulin locus as described herein.

[00236] In one aspect, a mouse or a cell made using a nucleic acid construct as described herein is provided.

[00237] In one aspect, a mouse made using a cell as described herein is provided. In one embodiment, the cell is a mouse ES cell.

[00238] 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.

[00239] In one aspect, a cell or tissue derived from a mouse as described herein is provided. In one embodiment, the cell or tissue is derived from spleen, lymph node or bone marrow of a mouse as described herein. In one embodiment, the cell is a B cell. In one embodiment the cell is an embryonic stem cell. In one embodiment, the cell is a germ cell.

[00240] In one embodiment, the tissue is selected from connective, muscle, nervous and epithelial tissue. In a specific embodiment, the tissue is reproductive tissue.

[00241] 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.

[00242] In aspect, use of cell or tissue derived from a mouse as described herein to make an antibody is provided. In one aspect, use of a cell or tissue derived from a mouse as described herein to make a hybridoma or quadroma is provided.

[00243] 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.

[00244] In one aspect, a nucleus derived from a mouse as described herein is provided. In one embodiment, the nucleus is from a diploid cell that is not a B cell.

[00245] 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 light chain gene segments. In a specific embodiment, the human immunoglobulin light chain gene segments are $V\kappa$ and/or $J\kappa$ gene segments that are operably linked to mouse heavy chain constant regions such that upon rearrangement encode a functional light chain variable domain fused to a mouse heavy chain constant domain.

[00246] In one aspect, a genetically modified mouse cell is provided; wherein the cell lacks a functional endogenous ADAM6 locus, and the cell comprises an ectopic nucleotide sequence that encodes a mouse ADAM6. In one embodiment, the cell further comprises a modification of an endogenous immunoglobulin heavy chain variable region sequence. In a specific embodiment, the modification of the endogenous immunoglobulin heavy chain variable region 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.

[00247] 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.

[00248] In one aspect, a mouse B cell is provided, wherein the mouse B cell comprises a rearranged immunoglobulin 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. In one embodiment, the rearranged immunoglobulin gene comprises a rearranged immunoglobulin light chain sequence contiguous with a heavy chain constant sequence. In one embodiment, the light chain sequence is a κ sequence; in one embodiment, the light chain sequence is a λ sequence.

[00249] 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 heavy chain immunoglobulin locus.

[00250] 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 heavy chain immunoglobulin locus.

[00251] In one embodiment, the mouse B cell comprises a rearranged non-mouse light chain immunoglobulin variable region sequence operably linked to a mouse or human heavy chain 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.

[00252] 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 one embodiment, the modified immunoglobulin heavy chain locus comprises an unrearranged immunoglobulin light chain gene segment operably linked to a heavy chain constant region sequence.

[00253] 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, wherein the mouse further comprises a modification comprising an unrearranged light chain immunoglobulin gene segment (a Vκ and/or Vλ and/or Vκ and Jκ and/or Vλ and Jλ) operably linked to a heavy chain constant region sequence. 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.

[00254] In one aspect, a pluripotent, induced pluripotent, or totipotent cell derived from a mouse as described herein is provided. In a specific embodiment, the cell is a mouse embryonic stem (ES) cell.

[00255] In one aspect, a cell is provided, comprising a modified immunoglobulin locus as described herein. In one embodiment, the cell is selected from a totipotent cell, a pluripotent cell, an induced pluripotent stem cell (iPS), and an ES cell. In a specific embodiment, the cell is a mouse cell, *e.g.*, a mouse ES cell. In one embodiment, the cell is homozygous for the modified immunoglobulin locus.

[00256] In one aspect, a cell is provided, comprising a nucleic acid sequence encoding a first polypeptide that comprises a first somatically mutated human Vκ or Vλ sequence fused to a human heavy chain constant region sequence.

[00257] In one embodiment, the cell further comprises a second polypeptide chain that comprises a second somatically mutated human Vκ or Vλ sequence fused to a human light chain constant region sequence.

[00258] In one embodiment, the human Vκ or Vλ sequence of the first polypeptide is cognate with the human Vκ or Vλ sequence of the second polypeptide.

[00259] In one embodiment, the Vκ or Vλ of the first polypeptide and the human Vκ or Vλ of the second polypeptide when associated specifically bind an antigen of interest. In a specific embodiment, the first polypeptide comprises a variable domain consisting essentially of a human Vκ, and the second polypeptide comprises a variable domain consisting of a human Vκ that is cognate with the human Vκ of the first polypeptide, and the human constant region sequence is an IgG sequence.

[00260] In one embodiment, the cell is selected from a CHO cell, a COS cell, a 293 cell, a HeLa cell, and a human retinal cell expressing a viral nucleic acid sequence (*e.g.*, a PERC.6™ cell.

[00261] In one aspect, a somatic rodent (*e.g.*, mouse) cell is provided, comprising a chromosome that comprises a genetic modification as described herein.

[00262] In one aspect, a rodent (*e.g.*, mouse) germ cell is provided, comprising a nucleic acid sequence that comprises a genetic modification as described herein.

[00263] In one aspect, a pluripotent, induced pluripotent, or totipotent cell derived from a rodent (*e.g.*, mouse) as described herein is provided. In a specific embodiment, the cell is a mouse embryonic stem (ES) cell.

[00264] In one aspect, use of a cell as described herein for the manufacture of a rodent (*e.g.*, a mouse), a cell, or a therapeutic protein (*e.g.*, an antibody or other antigen-binding protein) is provided. In one embodiment, use of a cell as described herein for the manufacture of a therapeutic protein is provided, wherein the therapeutic protein comprises a human variable domain. In a specific embodiment, the human variable domain comprises rearranged $V\kappa$ and $J\kappa$ gene segments.

[00265] In one aspect, a rodent (*e.g.*, a mouse) made using a targeting vector, nucleotide construct, or cell as described herein is provided.

[00266] In one aspect, use of a targeting vector as described herein for the manufacture of a rodent (*e.g.*, a mouse) or a cell (*e.g.,* a mouse ES cell, a mouse fibroblast, *etc.*) is provided. In one embodiment, the targeting vector comprises a human genomic fragment that contains unrearranged immunoglobulin gene segments. In a specific embodiment, the unrearranged immunoglobulin gene segments include V and J gene segments. In a specific embodiment, the unrearranged immunoglobulin gene segments include V, D and J gene segments.

[00267] 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.

[00268] 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.

[00269] 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.

[00270] 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.

[00271] In one aspect, a nucleic acid construct is provided that comprises a human D_H gene segment juxtaposed upstream and downstream with a 23-mer spaced RSS. In a specific embodiment, the nucleic acid construct comprises a homology arm that is homologous to a human genomic sequence comprising human $V\kappa$ gene segments. In one embodiment, the targeting construct comprises all or substantially all human D_H gene segments each juxtaposed upstream and downstream with a 23-mer spaced RSS.

[00272] In one aspect, a nucleic acid construct is provided that comprises a human $J\kappa$ gene segment juxtaposed upstream with a 12-mer spaced RSS. In a specific embodiment, the nucleic acid construct comprises a first homology arm that contains homology to a human genomic D_H gene sequence that is juxtaposed upstream and downstream with a 23-mer spaced RSS. In one embodiment, the nucleic acid construct comprises a second homology arm that contains homology to a human genomic J gene sequence or that contains homology to a mouse heavy chain constant region sequence or that contains homology to a J-C intergenic sequence upstream of a mouse constant region heavy chain sequence.

[00273] In one aspect, a nucleic acid construct is provided that comprises a human $V\lambda$ segment juxtaposed downstream with a 23-mer spaced RSS, a human D_H segment juxtaposed upstream and downstream with a 12-mer spaced RSS, and a human J segment selected from a $J\kappa$ segment juxtaposed upstream with a 23-mer spaced RSS, a human $J\lambda$ segment juxtaposed upstream with a 23-mer spaced RSS, and a human J_H segment juxtaposed upstream with a 23-mer spaced RSS. In one embodiment, the construct comprises a homology arm that contains homology to a mouse constant region sequence, a J-C intergenic mouse sequence, and/or a human $V\lambda$ sequence.

[00274] In one embodiment, the nucleic acid construct comprises a human λ light chain variable region sequence that comprises a fragment of cluster A of the human λ light chain locus. In a specific embodiment, the fragment of cluster A of the human λ light chain locus extends from hVλ3-27 through hVλ3-1.

[00275] In one embodiment, the nucleic acid construct comprises a human λ light chain variable region sequence that comprises a fragment of cluster B of the human λ light chain locus. In a specific embodiment, the fragment of cluster B of the human λ light chain locus extends from hVλ5-52 through hVλ1-40.

[00276] In one embodiment, nucleic acid construct comprises a human λ light chain variable region sequence that comprises a genomic fragment of cluster A and a genomic fragment of cluster B. In a one embodiment, the human λ light chain variable region sequence comprises at least one gene segment of cluster A and at least one gene segment of cluster B.

[00277] In one embodiment, the human λ light chain variable region sequence comprises at least one gene segment of cluster B and at least one gene segment of cluster C.

[00278] In one aspect, a nucleic acid construct is provided, comprising a human D_H segment juxtaposed upstream and downstream with a 23-mer spaced RSS normally found in nature flanking either a Jκ, a J_H, a Vλ, or a V_H segment. In one embodiment, the nucleic acid construct comprises a first homology arm homologous to a human V-J intergenic region or homologous to a human genomic sequence comprising a human V gene segment. In one embodiment, the nucleic acid construct comprises a second homology arm homologous to a human or mouse heavy chain constant region sequence. In a specific embodiment, the human or mouse heavy chain constant region sequence is selected from a C_H1, hinge, C_H2, C_H3, and a combination thereof. In one embodiment, the nucleic acid construct comprises a human J gene segment flanked upstream with a 12-mer RSS. In one embodiment, the nucleic acid construct comprises a second homology arm that contains homology to a J gene segment flanked upstream with a 12-mer RSS. In one embodiment, the J gene segment is selected from a human Jκ, a human Jλ, and a human J_H.

[00279] In one aspect, a nucleic acid construct is provided that comprises a human D_H segment juxtaposed upstream and downstream with a 23-mer spaced RSS, and a site-specific recombinase recognition sequence, *e.g.*, a sequence recognized by a site-specific recombinase such as a Cre, a Flp, or a Dre protein.

[00280] In one aspect, a nucleic acid construct is provided that comprises a human Vλ or a human Vκ segment, a D_H segment juxtaposed upstream and downstream with a 12-mer or a 23-mer spaced RSS, and a human J segment with a 12-mer or a 23-mer spaced RSS,

wherein the 12-mer or 23-mer spaced RSS is positioned immediately 5' to the human J segment (*i.e.,* with respect to the direction of transcription). In one embodiment, the construct comprises a human Vλ juxtaposed with a 3' 23-mer spaced RSS, a human D_H segment juxtaposed upstream and downstream with a 12-mer spaced RSS, and a human Jκ segment juxtaposed with a 5' 23-mer spaced RSS. In one embodiment, the construct comprises a human Vκ juxtaposed with a 3' 12-mer spaced RSS, a human D_H segment juxtaposed upstream and downstream with a 23-mer spaced RSS, and a human Jλ segment juxtaposed with a 5' 12-mer spaced RSS.

[00281] In one aspect, a targeting vector is provided, comprising (a) a first targeting arm and a second targeting arm, wherein the first and second targeting arms are independently selected from human and mouse targeting arms, wherein the targeting arms direct the vector to an endogenous or modified immunoglobulin V region gene locus; and, (b) a contiguous sequence of human V_L gene segments or a contiguous sequence of human V_L gene segments and at least one human Jκ gene segment, wherein the contiguous sequence is selected from the group consisting of (i) hVκ4-1 through hVκ1-6 and Jκ1, (ii) hVκ4-1 through hVκ1-6 and Jκ1 through Jκ2, (iii) hVκ4-1 through hVκ1-6 and Jκ1 through Jκ3, (iv) hVκ4-1 through hVκ1-6 and Jκ1 through Jκ4, (v) hVκ4-1 through hVκ1-6 and Jκ1 through Jκ5, (vi) hVκ3-7 through hVκ1-16, (vii) hVκ1-17 through hVκ2-30, (viii) hVκ3-31 through hVκ2-40, and (ix) a combination thereof.

[00282] In one embodiment, the targeting arms that direct the vector to an endogenous or modified immunoglobulin locus are identical or substantially identical to a sequence at the endogenous or modified immunoglobulin locus.

[00283] In one aspect, a method for making a genetically modified mouse 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 mouse with one or more human immunoglobulin light chain and/or 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 mouse 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 mouse 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 mouse 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 mouse 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 mouse 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 mouse include V, D and J gene segments.

[00284] In one aspect, a method for making a genetically modified mouse 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 mouse with one or more human immunoglobulin light 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 mouse with one or more human immunoglobulin light chain 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 mouse 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 mouse include V 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 mouse 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 mouse include V 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 mouse include V gene segments.

[00285] In a specific embodiment, the V gene segments are V_L gene segments. In another specific embodiment, the J gene segments are J_L gene segments.

[00286] 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 mouse 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.

[00287] In one aspect, a method for making a genetically modified mouse is provided, comprising replacing a mouse nucleotide sequence that comprises a mouse immunoglobulin gene segment and a mouse ADAM6 (or ortholog or homolog or fragment thereof functional in a male mouse) nucleotide sequence with a sequence comprising a human immunoglobulin gene segment to form a first chimeric locus, then inserting a sequence comprising a mouse 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.

[00288] In one embodiment, the second chimeric locus comprises a human immunoglobulin heavy chain variable gene segment (V_H). In one embodiment, the second chimeric locus comprises a human immunoglobulin light chain variable gene segment (V_L). 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 specific embodiment, the second chimeric locus comprises a human V_L gene segment operably linked to a human J_H or a human J_L 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.

[00289] In one aspect, a method is provided for modifying a heavy chain immunoglobulin locus of a mouse, comprising: (a) making a first modification of a mouse heavy chain immunoglobulin locus that results in a reduction or elimination of endogenous mouse ADAM6 activity in a male mouse; and, (b) making a second modification to add a nucleic acid sequence that confers upon the mouse ADAM6 activity that is functional in a male mouse.

[00290] In one embodiment, the first modification comprises the addition of a human immunoglobulin sequence or the replacement of a mouse immunoglobulin sequence with a human immunoglobulin sequence.

[00291] In one embodiment, the human immunoglobulin sequence is a heavy chain sequence. In one embodiment, the human immunoglobulin sequence is a light chain sequence.

[00292] In one embodiment, the first and the second modification are made in a single ES cell, and the single ES cell is introduced into a host embryo to make the mouse.

[00293] In one aspect, a progeny of a mating of a mouse as described herein with a second mouse that is a wild-type mouse or genetically modified is provided.

[00294] 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 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.

[00295] In one aspect, use of a mouse as described herein to introduce an ectopic ADAM6 sequence into a mouse that lacks a functional endogenous ADAM6 sequence is provided, wherein the use comprises mating a mouse as described herein with the mouse that lacks the functional endogenous ADAM6 sequence.

[00296] 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.

[00297] 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 heavy chain immunoglobulin 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.

[00298] 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.

[00299] 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.

[00300] 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.

[00301] In one aspect, a method for making a genetically modified mouse is provided, comprising replacing at an endogenous heavy chain locus one or more immunoglobulin heavy chain gene segments of a mouse with one or more human immunoglobulin light chain gene segments. In one embodiment, the replacement is of all or substantially all functional mouse immunoglobulin heavy chain segments (*i.e.*, V_H, D_H, and J_H segments) with one or more functional human light chain segments (*i.e.*, V_L and J_L segments). In one embodiment, the replacement is of all or substantially all functional mouse heavy chain V_H, D_H, and J_H segments with all or substantially all human Vλ or Vκ segments and at least one Jλ or Jκ segment. In a specific embodiment, the replacement includes all or substantially all functional human Jλ or Jκ segments.

[00302] In one aspect, a method is provided for making a mouse that expresses a polypeptide that comprises a sequence derived from a human immunoglobulin Vλ or Vκ and/or Jλ or Jκ segment fused with a mouse heavy chain constant region, comprising replacing endogenous heavy chain immunoglobulin variable segments (V_H, D_H, and J_H) with at least one human Vλ or Vκ segment and at least one human Jλ or Jκ segment, wherein the replacement is in a pluripotent, induced pluripotent, or totipotent mouse cell to form a genetically modified mouse progenitor cell; the genetically modified mouse progenitor cell is introduced into a mouse host; and, the mouse host comprising the genetically modified progenitor cell is gestated to form a mouse comprising a genome derived from the genetically modified mouse 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.

[00303] In one aspect, a method is provided for making a genetically modified mouse as described herein, comprising introducing by nuclear transfer a nucleic acid containing a modification as described herein into a cell, and maintaining the cell under suitable conditions (*e.g.*, including culturing the cell and gestating an embryo comprising the cell in a surrogate mother) to develop into a mouse as described herein.

[00304] In one aspect, a method for making a modified mouse is provided, comprising modifying as described herein a mouse ES cell or pluripotent or totipotent or induced pluripotent mouse cell to include one or more unrearranged immunoglobulin light chain

variable gene segments operably linked to an immunoglobulin heavy chain constant sequence, culturing the ES cell, introducing the cultured ES cell into a host embryo to form a chimeric embryo, and introducing the chimeric embryo into a suitable host mouse to develop into a modified mouse. In one embodiment, the one or more unrearranged immunoglobulin light chain variable region gene segments are human λ or human κ gene segments. In one embodiment, the one or more unrearranged immunoglobulin light chain variable region gene segments comprise human Vλ or human Vκ segments and one or more Jλ, Jκ, or J_H segments. In one embodiment, the heavy chain constant gene sequence is a human sequence selected from C_H1, hinge, C_H2, C_H3, and a combination thereof. In one embodiment, the one or more unrearranged immunoglobulin light chain variable gene segments replace all or substantially all functional endogenous heavy chain variable region gene segments at the endogenous heavy chain locus, and the heavy chain constant sequence is a mouse sequence comprising a C_H1, a hinge, a C_H2, and a C_H3.

[00305] In one aspect, nucleic acids constructs, cells, embryos, mice, and methods are provided for making proteins that comprise one or more κ and/or λ light chain variable region immunoglobulin sequences and an immunoglobulin heavy chain constant region sequence, including proteins that comprise a human λ or κ light chain variable domain and a human or mouse heavy chain constant region sequence.

[00306] In one aspect, a nucleotide sequence encoding an immunoglobulin variable region made in a mouse as described herein is provided.

[00307] In one aspect, a heavy chain or light chain variable region amino acid sequence of an antibody made in a mouse as described herein is provided.

[00308] In one aspect, a heavy chain or light chain variable region nucleotide sequence encoding a variable region of an antibody made in a mouse as described herein is provided.

[00309] In one aspect, an antibody or antigen-binding fragment thereof (*e.g.*, Fab, $F(ab)_2$, scFv) made in a mouse as described herein is provided.

[00310] In one aspect, binding proteins are described that comprise immunoglobulin variable domains that are derived from light chain (*i.e.*, kappa (κ) and/or lambda (λ)) immunoglobulin variable domains, but not from full-length heavy chain immunoglobulin variable domains. Methods and compositions for making binding proteins, including genetically modified mice, are also provided.

[00311] In one aspect, a method is provided for making an antigen-binding protein that does not comprise an immunoglobulin heavy chain variable domain, comprising a step of immunizing a mouse as described herein with an antigen of interest, and maintaining the mouse under conditions that allow it to make an antigen-binding protein that specifically binds the antigen of interest.

[00312] In one aspect, a method is provided for making an antigen-binding protein that comprises a first immunoglobulin light chain variable domain contiguous with a heavy chain constant regions sequence, and a second immunoglobulin light chain variable domain that is contiguous with a light chain constant sequence, comprising a step of immunizing a mouse as described herein with an antigen of interest, and maintaining the mouse under conditions that allow it to make an antigen-binding protein that specifically binds the antigen of interest.

[00313] In one aspect, a method is provided for making an antigen-binding protein that comprises an immunoglobulin light chain variable domain contiguous with a heavy chain constant regions sequence, wherein the antigen-binding protein lacks an immunoglobulin light chain comprising a step of immunizing a mouse as described herein that comprises a knockout of an endogenous κ and λ locus (or that comprises a nonfunctional endogenous κ and/or λ locus) with an antigen of interest, and maintaining the mouse under conditions that allow it to make an antigen-binding protein that specifically binds the antigen of interest.

[00314] In one aspect, use of a mouse as described herein to make an immunoglobulin variable region nucleotide sequence is provided. In one embodiment, the variable region nucleotide sequence comprises rearranged V_L, D_H and J_L gene segments. In one embodiment, the variable region nucleotide sequence comprises rearranged V_L, D_H and J_H gene segments. In one embodiment, the variable region nucleotide sequence comprises rearranged Vκ and Jκ gene segments. In one embodiment, the variable region nucleotide sequence comprises rearranged Vλ and Jλ gene segments.

[00315] 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. In one embodiment, the fully human Fab or a fully human $F(ab)_2$ comprises rearranged V_L, D_H and J_L gene segments. In one embodiment, the fully human Fab or a fully human $F(ab)_2$ comprises rearranged V_L, D_H and J_H gene segments. In one embodiment, the fully human Fab or a fully human $F(ab)_2$ comprises rearranged Vκ and Jκ gene segments. In one embodiment, the fully human Fab or a fully human $F(ab)_2$ comprises rearranged Vλ and Jλ gene segments.

[00316] In one aspect, use of a mouse as described herein to make a fully human Fab (comprising a first human V_L fused with a human light chain constant region, and a second human V_L fused with a human heavy chain constant region sequence) or a fully human $F(ab)_2$ is provided.

[00317] In one aspect, use of a mouse as described herein to make an immortalized cell line is provided. In one embodiment, the immortalized cell line comprises a nucleic acid sequence encoding a human Vλ or Vκ domain operably linked to a nucleic acid sequence that comprises a mouse constant region nucleic acid sequence. In one embodiment, the

immortalized cell line expresses an antibody that comprises a human variable domain. In a specific embodiment, the human variable domain is a light chain variable domain.

[00318] In one aspect, use of a mouse as described herein to make a hybridoma or quadroma is provided. In one embodiment, the hybridoma or quadroma expresses a polypeptide comprising a human variable domain that binds an antigen of interest.

[00319] In one aspect, use of a mouse as described herein to make a phage library containing human light chain variable regions is provided. In one embodiment, the light chain variable regions are human Vκ regions.

[00320] In one aspect, use of a mouse as described herein to generate a variable region sequence for making a human antigen-binding protein 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 human light chain variable region nucleic acid sequences from the lymphocyte thereby generating a variable region sequence.

[00321] 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.

[00322] 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.

[00323] In one embodiment, the lymphocyte is a B cell.

[00324] In one embodiment, the one or more variable region nucleic acid sequences 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 is a λ light chain variable region sequence.

[00325] In one embodiment, use of a mouse as described herein to generate one or more κ light chain variable region sequences for making a human antigen-binding protein 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 κ light chain variable region nucleic acid sequence from the B lymphocyte thereby generating the κ light chain variable region sequence.

[00326] In one embodiment, use of a mouse as described herein to generate a κ light chain variable region sequence for making a human antigen-binding protein 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 κ light chain variable region nucleic acid sequence from the B lymphocyte thereby generating κ light chain variable region sequence.

[00327] In one embodiment, use of a mouse as described herein to generate a κ light chain variable region sequence for making a human antigen-binding protein 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 κ light chain variable region nucleic acid sequence from the B lymphocyte thereby generating the κ light chain variable region sequence. In various embodiments, the one or more labeled antibodies are selected from an IgM, an IgG, and/or a combination thereof.

[00328] In various embodiments, use of a mouse as described herein to generate a κ light chain variable region sequence for making a human antigen-binding protein is provided, further comprising fusing the amplified κ light chain variable region sequence to human heavy or light chain constant region sequences or optionally a human heavy chain variable region seuqence, expressing the fused sequences in a cell, and recovering the expressed sequences thereby generating a human antigen-binding protein.

[00329] 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.

[00330] In one aspect, a method for generating a human light chain variable region 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 human light chain variable region specific against the antigen, generating at least one cell producing a human antigen-binding protein comprising the light chain variable region specific against the antigen, and culturing at least one cell producing the human antigen-binding protein, and obtaining said human antigen-binding protein. In one embodiment, the human light chain variable region is a human V_{κ} region.

[00331] In various embodiments, the at least one cell isolated from the mouse producing a human light chain variable region specific against the antigen is a splenocyte or a B cell.

[00332] In various embodiments, the antigen-binding protein is an antibody.

[00333] 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.

[00334] 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 multispecific antibody (*e.g.*, a bispecific antibody), an scFv, a bis-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).

[00335] 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.

[00336] In one aspect, use of a mouse as described herein to make a nucleic acid sequence encoding a first human light chain immunoglobulin variable sequence (V_L1) that is cognate with a second human light chain immunoglobulin variable sequence (V_L2), wherein the V_L1 fused with a human immunoglobulin light chain constant region (polypeptide 1) expresses with V_L2 fused with a human immunoglobulin heavy chain constant region (polypeptide 2), as a dimer of polypeptide1/polypeptide 2, to form a V_L1-V_L2 antibody.

[00337] In one aspect, use of a mouse as described herein to make a nucleic acid sequence encoding a human immunoglobulin light chain variable sequence that is fused with a human immunoglobulin heavy chain sequence, wherein the nucleic acid sequence encodes a human V_L-C_H polypeptide, wherein the human V_L-C_H polypeptide expresses as a dimer, and wherein the dimer expresses in the absence of an immunoglobulin light chain (*e.g.*, in the absence of a human λ or human κ light chain). In one embodiment, the V_L-C_H

dimer specifically binds an antigen of interest in the absence of a λ light chain and in the absence of a κ light chain.

[00338] In one aspect, use of a mouse as described herein to make a nucleic acid sequence encoding all or a portion of an immunoglobulin variable domain. In one embodiment, the immunoglobulin variable domain is a human Vλ or human Vκ domain.

[00339] In one aspect, use of a nucleic acid construct as described herein for the manufacture of a mouse, a cell, or a therapeutic protein (*e.g.*, an antibody or other antigen-binding protein) is provided.

[00340] In one aspect, use of a nucleic acid sequence from a mouse as described herein to make a cell line for the manufacture of a human therapeutic is provided. In one embodiment, the human therapeutic is a binding protein comprising a human light chain variable sequence (*e.g.*, derived from a human Vλ or human Vκ segment) fused with a human heavy chain constant sequence. In one embodiment, the human therapeutic comprises a first polypeptide that is a human λ or κ immunoglobulin light chain, and a second polypeptide that comprises a human Vλ or human Vκ variable sequence fused with a human heavy chain constant sequence.

[00341] In one aspect, an expression system is provided, comprising a mammalian cell transfected with a DNA construct that encodes a polypeptide that comprises a somatically mutated human V_L domain fused with a human C_H domain.

[00342] In one embodiment, the expression system further comprises a nucleotide sequence that encodes an immunoglobulin V_L domain fused with a human C_L domain, wherein the V_L domain fused with the human C_L domain is a cognate light chain with the V_L domain fused with the human C_H domain.

[00343] In one embodiment, the mammalian cell is selected from a CHO cell, a COS cell, a Vero cell, a 293 cell, and a retinal cell that expresses a viral gene (*e.g.*, a PER.C6™ cell).

[00344] In one aspect, a method for making a binding protein is provided, comprising obtaining a nucleotide sequence encoding a V_L domain from a gene encoding a V_L region fused to a C_H region from a cell of a mouse as described herein, and cloning the nucleotide sequence encoding the V_L region sequence in frame with a gene encoding a human C_H region to form a human binding protein sequence, expressing the human binding protein sequence in a suitable cell.

[00345] In one embodiment, the mouse has been immunized with an antigen of interest, and the V_L region fused to the C_H region specifically binds (*e.g.*, with a K_D in the micromolar, nanomolar, or picomolar range) an epitope of the antigen of interest. In one embodiment,

nucleotide sequence encoding the V_L region fused to the C_H region is somatically mutated in the mouse.

[00346] In one embodiment, the suitable cell is selected from a B cell, a hybridoma, a quadroma, a CHO cell, a COS cell, a 293 cell, a HeLa cell, and a human retinal cell expressing a viral nucleic acid sequence (*e.g.,* a PERC.6™ cell).

[00347] In one embodiment, the C_H region comprises a human IgG isotype. In a specific embodiment, the human IgG is selected from an IgG1, IgG2, and IgG4. In another specific embodiment, the human IgG is IgG1. In another specific embodiment, the human IgG is IgG4. In another specific embodiment, the human IgG4 is a modified IgG4. In one embodiment, the modified IgG4 comprises a substitution in the hinge region. In a specific embodiment, the modified IgG4 comprises a substitution at amino acid residue 228 relative to a wild-type human IgG4, numbered according to the EU numbering index of Kabat. In a specific embodiment, the substitution at amino acid residue 228 is a S228P substitution, numbered according to the EU numbering index of Kabat.

[00348] In one embodiment, the cell further comprises a nucleotide sequence encoding a V_L domain from a light chain that is cognate to the V_L domain fused to the C_H region, and the method further comprises expressing the nucleotide sequence encoding the cognate V_L domain fused to a human $C\kappa$ or $C\lambda$ domain.

[00349] In one aspect, a method for making a bispecific antigen-binding protein is provided, comprising exposing a first mouse as described herein to a first antigen of interest and identifying a sequence of a first human V_L domain that specifically binds the first antigen of interest; exposing a second mouse as described herein to a second antigen of interest and identifying a sequence of a second human V_L domain that specifically binds the second antigen of interest; wherein the first human V_L domain does not bind the second antigen of interest, and the second human V_L domain does not bind the first antigen of interest; and fusing the sequence of the first human V_L domain to a first heavy chain constant sequence to form a first antigen-binding polypeptide, and fusing the sequence of the second human V_L domain to a second heavy chain constant sequence to form a second antigen-binding polypeptide; and, employing the first and the second antigen-binding polypeptides in a bispecific binding protein.

[00350] In one embodiment, the antigen-binding protein further comprises a first immunoglobulin light chain that comprises a human κ or λ variable domain that is cognate with the first human V_L domain, and a second immunoglobulin light chain that comprises a human κ or λ variable domain that is cognate with the second human V_L domain.

[00351] In one embodiment, the first heavy chain constant sequence is identical to the second heavy chain constant sequence. In one embodiment, the first heavy chain constant

sequence comprises a modification that reduces or eliminates binding of the first heavy chain constant region to protein A, and the second heavy chain constant sequence binds protein A.

[00352] In one embodiment, the first and second human V_L domains comprise a sequence derived from a human Vκ and a human Jκ gene segment. In one embodiment, the first and second human V_L domains comprise a sequence derived from a human Vλ and a human Jλ gene segment. In one embodiment, the first and second human V_L domains comprise a sequence derived from a human D_H gene segment. In one embodiment, the first and second human V_L domains comprise a sequence derived from a human J_H gene segment. In a specific embodiment, the first and second human V_L domains comprise a sequence derived from a human D_H and a human J_H gene segment. In a specific embodiment, the first and second human V_L domains comprise a sequence derived from a human D_H and a human Jκ gene segment.

[00353] In one embodiment, the first human V_L domain comprises a sequence derived from a human Vκ and a human Jκ gene segment and the second human V_L domain comprises a sequence derived from a human Vλ and a human Jλ gene segment. In one embodiment, the first human V_L domain comprises a sequence derived from a human Vλ and a human Jλ gene segment and the second human V_L domain comprises a sequence derived from a human Vκ and a human Jκ gene segment.

[00354] In one aspect, an immunoglobulin variable region (VR) (*e.g.*, comprising a human V_L sequence fused with a human J_L, or J_H, or D_H and J_H, or D_H and J_L) made in a mouse as described herein is provided. In a specific embodiment, the immunoglobulin VR is derived from a germline human gene segment selected from a Vκ segment and a Vλ segment, wherein the VR is encoded by a rearranged sequence from the mouse wherein the rearranged sequence is somatically hypermutated. In one embodiment, the rearranged sequence comprises 1 to 5 somatic hypermutations. In one embodiment, the rearranged sequence comprises at least 6, 7, 8, 9, or 10 somatic hypermutations. In one embodiment, the rearranged sequence comprises more than 10 somatic hypermutations. In one embodiment, the rearranged sequence is fused with one or more human or mouse heavy chain constant region sequences (*e.g.*, selected from a human or mouse C_H1, hinge, C_H2, C_H3, and a combination thereof).

[00355] In one aspect, an immunoglobulin variable domain amino acid sequence of a binding protein made in a mouse as described herein is provided. In one embodiment, the VR is fused with one or more human or mouse heavy chain constant region sequences (*e.g.*, selected from a human or mouse C_H1, hinge, C_H2, C_H3, and a combination thereof).

[00356] In one aspect, a light chain variable domain encoded by a nucleic acid sequence derived from a mouse as described herein is provided.

[00357] In one aspect, an antibody or antigen-binding fragment thereof (*e.g.*, Fab, $F(ab)_2$, scFv) made in a mouse as described herein, or derived from a sequence made in a mouse as described herein, is provided.

BRIEF DESCRIPTION OF THE FIGURES

[00358] **FIG. 1** illustrates schematics (not to scale) of the mouse heavy chain locus (top) and the human κ light chain locus (bottom). The mouse heavy chain locus is about 3 Mb in length and contains approximately 200 heavy chain variable (V_H) gene segments, 13 heavy chain diversity (D_H) gene segments and 4 heavy chain joining (J_H) gene segments as well as enhancers (Enh) and heavy chain constant (C_H) regions. The human κ light chain locus is duplicated into distal and proximal contigs of opposite polarity spanning about 440 kb and 600 kb, respectively. Between the two contigs is about 800 kb of DNA that is believed to be free of Vκ gene segments. The human κ light chain locus contains about 76 Vκ gene segments, 5 Jκ gene segments, an intronic enhancer (Enh) and a single constant region (Cκ).

[00359] **FIG. 2** shows an exemplary targeting strategy for progressive insertion of 40 human Vκ and five human Jκ gene segments into the mouse heavy chain locus resulting in a modified mouse immunoglobulin heavy chain locus comprising human Vκ and Jκ gene segments operably linked to mouse immunoglobulin heavy chain constant regions. Hygromycin (hyg) and neomycin (neo) selection cassettes are shown with recombinase recognition sites (R1, R2, etc.).

[00360] **FIG. 3** shows an exemplary targeting strategy for progressive insertion of a plurality of human Vλ and a single human Jλ gene segment into the mouse heavy chain locus. Hygromycin (hyg) and neomycin (neo) selection cassettes are shown with recombinase recognition sites (R1, R2, etc.).

[00361] **FIG. 4** shows an exemplary targeting strategy for progressive insertion of a plurality of human Vλ and four human Jλ gene segments into the mouse heavy chain locus. Hygromycin (hyg) and neomycin (neo) selection cassettes are shown with recombinase recognition sites (R1, R2, etc.).

[00362] **FIG. 5** shows an exemplary targeting strategy for progressive insertion of human Vλ, human D_H and human J_H gene segments into the mouse heavy chain locus. Hygromycin (hyg) and neomycin (neo) selection cassettes are shown with recombinase recognition sites (R1, R2, etc.).

[00363] **FIG. 6** shows an exemplary targeting strategy for progressive insertion of human Vλ, human D_H and human Jκ gene segments into the mouse heavy chain locus. Hygromycin (hyg) and neomycin (neo) selection cassettes are shown with recombinase recognition sites (R1, R2, etc.).

[00364] **FIG. 7** shows the steps for cloning a genomic fragment encoding mouse ADAM6 genes from a mouse immunoglobulin heavy chain V-D intergenic region and engineering steps to modify the genomic fragment for insertion into a modified immunoglobulin heavy chain locus.

[00365] **FIG. 8** shows a targeting strategy for insertion of a genomic fragment encoding mouse ADAM6 genes into the Vκ-Jκ intergenic region of a modified mouse immunoglobulin heavy chain locus containing human Vκ and Jκ gene segments operably linked to mouse immunoglobulin heavy chain constant regions.

[00366] **FIG. 9** shows a targeting strategy for insertion of a genomic fragment encoding mouse ADAM6 genes upstream (5') of human Vκ gene segments (*i.e.,* hVκ2-40) of a modified mouse immunoglobulin heavy chain locus containing human Vκ and Jκ gene segments operably linked to mouse immunoglobulin heavy chain constant regions.

DETAILED DESCRIPTION

[00367] 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.

[00368] Unless defined otherwise, all terms and phrases used herein include the meanings that the terms and phrases have attained in the art, unless the contrary is clearly indicated or clearly apparent from the context in which the term or phrase is used. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, particular methods and materials are now described. All publications mentioned are hereby incorporated by reference.

[00369] 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.

[00370] 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.

[00371] The term "contiguous" includes reference to occurrence on the same nucleic acid molecule, *e.g.,* two nucleic acid sequences are "contiguous" if they occur on the same nucleic molecule but are interrupted by another nucleic acid sequence. For example, a rearranged V(D)J sequence is "contiguous" with a constant region gene sequence, although the final codon of the V(D)J sequence is not followed immediately by the first codon of the constant region sequence. In another example, two V gene segment sequences are "contiguous" if they occur on the same genomic fragment, although they may be separated by sequence that does not encode a codon of the V region, *e.g.*, they may be separated by a regulatory sequence, *e.g.*, a promoter or other noncoding sequence. In one embodiment, a contiguous sequence includes a genomic fragment that contains genomic sequences arranged as found in a wild-type genome.

[00372] The phrase "derived from" when used concerning a variable region "derived from" a cited gene or gene segment includes the ability to trace the sequence back to a particular unrearranged gene segment or gene segments that were rearranged to form a gene that expresses the variable domain (accounting for, where applicable, splice differences and somatic mutations).

[00373] The phrase "functional" when used concerning a variable region gene segment or joining gene segment refers to usage in an expressed antibody repertoire; *e.g.*, in humans

Vλ gene segments 3-1, 4-3, 2-8, *etc.* are functional, whereas Vλ gene segments 3-2, 3-4, 2-5, *etc.* are nonfunctional.

[00374] A "heavy chain locus" includes a location on a chromosome, *e.g.*, a mouse chromosome, wherein in a wild-type mouse heavy chain variable (V_H) , heavy chain diversity (D_H), heavy chain joining (J_H), and heavy chain constant (C_H) region DNA sequences are found.

[00375] The phrase "bispecific binding protein" includes a binding protein capable of selectively binding two or more epitopes. Bispecific binding proteins comprise two different polypeptides that comprise a first light chain variable domain (V_L1) fused with a first C_H region and a second light chain variable domain (V_L2) fused with a second C_H region. In general, the first and the second C_H regions are identical, or they differ by one or more amino acid substitutions (*e.g.*, as described herein). V_L1 and V_L2 specifically binding different epitopes—either on two different molecules (*e.g.,* antigens) or on the same molecule (*e.g.,* on the same antigen). If a bispecific binding protein selectively binds two different epitopes (a first epitope and a second epitope), the affinity of V_L1 for the first epitope will generally be at least one to two or three or four orders of magnitude lower than the affinity of V_L1 for the second epitope, and vice versa with respect to V_L2. The epitopes recognized by the bispecific binding protein can be on the same or a different target (*e.g.,* on the same or a different antigen). Bispecific binding proteins can be made, for example, by combining a V_L1 and a V_L2 that recognize different epitopes of the same antigen. For example, nucleic acid sequences encoding V_L sequences that recognize different epitopes of the same antigen can be fused to nucleic acid sequences encoding different C_H regions, and such sequences can be expressed in a cell that expresses an immunoglobulin light chain, or can be expressed in a cell that does not express an immunoglobulin light chain. A typical bispecific binding protein has two heavy chains each having three light chain CDRs, followed by (N-terminal to C-terminal) a C_H1 domain, a hinge, a C_H2 domain, and a C_H3 domain, and an immunoglobulin light chain that either does not confer antigen-binding specificity but that can associate with each heavy chain, or that can associate with each heavy chain and that can bind one or more of the epitopes bound by V_L1 and/or V_L2, or that can associate with each heavy chain and enable binding or assist in binding of one or both of the heavy chains to one or both epitopes.

[00376] Therefore, two general types of bispecific binding proteins are (1) V_L1-C_H (dimer), and (2) V_L1-C_H:light chain + V_L2-C_H:light chain, wherein the light chain is the same or different. In either case, the C_H (*i.e.*, the heavy chain constant region) can be differentially modified (*e.g.*, to differentially bind protein A, to increase serum half-life, *etc.*) as described herein, or can be the same.

[00377] The term "cell," when used in connection with expressing a sequence includes any cell that is suitable for expressing a recombinant nucleic acid sequence. Cells include those of prokaryotes and eukaryotes (single-cell or multiple-cell), bacterial cells (*e.g.,* strains of *E. coli, Bacillus spp., Streptomyces spp., etc.),* mycobacteria cells, fungal cells, yeast cells (*e.g., S. cerevisiae, S. pombe, P. pastoris, P. methanolica, etc.*), plant cells, insect cells (*e.g., SF-9, SF-21, baculovirus-infected insect cells, Trichoplusia ni, etc.*), non-human animal cells, human cells, B cells, or cell fusions such as, for example, hybridomas or quadromas. In some embodiments, the cell is a human, monkey, ape, hamster, rat, or mouse cell. In some embodiments, the cell is eukaryotic and is selected from the following cells: CHO (*e.g.,* CHO K1, DXB-11 CHO, Veggie-CHO), COS (*e.g.,* COS-7), retinal cell, Vero, CV1, kidney (*e.g.,* HEK293, 293 EBNA, MSR 293, MDCK, HaK, BHK), HeLa, HepG2, WI38, MRC 5, Colo205, HB 8065, HL-60, (*e.g.,* BHK21), Jurkat, Daudi, A431 (epidermal), CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT 060562, Sertoli cell, BRL 3A cell, HT1080 cell, myeloma cell, tumor cell, and a cell line derived from an aforementioned cell. In some embodiments, the cell comprises one or more viral genes, *e.g.* a retinal cell that expresses a viral gene (*e.g.,* a PER.C6™ cell).

[00378] The term "cognate," when used in the sense of "cognate with," *e.g.*, a first V_L domain that is "cognate with" a second V_L domain, is intended to include reference to the relation between two V_L domains from a same binding protein made by a mouse in accordance with the invention. For example, a mouse that is genetically modified in accordance with an embodiment of the invention, *e.g.*, a mouse having a heavy chain locus in which V_H, D_H, and J_H regions are replaced with V_L and J_L regions, makes antibody-like binding proteins that have two identical polypeptide chains made of the same mouse C_H region (*e.g.*, an IgG isotype) fused with a first human V_L domain, and two identical polypeptide chains made of the same mouse C_L region fused with a second human V_L domain. During clonal selection in the mouse, the first and the second human V_L domains were selected by the clonal selection process to appear together in the context of a single antibody-like binding protein. Thus, first and second V_L domains that appear together, as the result of the clonal selection process, in a single antibody-like molecule are referred to as being "cognate." In contrast, a V_L domain that appears in a first antibody-like molecule and a V_L domain that appears in a second antibody-like molecule are *not* cognate, *unless* the first and the second antibody-like molecules have identical heavy chains (*i.e.*, unless the V_L domain fused to the first human heavy chain region and the V_L domain fused to the second human heavy chain region are identical).

[00379] The phrase "complementarity determining region," or the term "CDR," includes an amino acid sequence encoded by a nucleic acid sequence of an organism's immunoglobulin

genes that normally (*i.e.,* in a wild-type animal) appears between two framework regions in a variable region of a light or a heavy chain of an immunoglobulin molecule (*e.g.,* an antibody or a T cell receptor). A CDR can be encoded by, for example, a germline sequence or a rearranged or unrearranged sequence, and, for example, by a naïve or a mature B cell or a T cell. In some circumstances (*e.g.,* for a CDR3), CDRs can be encoded by two or more sequences (*e.g.,* germline sequences) that are not contiguous (*e.g.,* in an unrearranged nucleic acid sequence) but are contiguous in a B cell nucleic acid sequence, *e.g.,* as the result of splicing or connecting the sequences (*e.g.,* V-D-J recombination to form a heavy chain CDR3).

[00380] The phrase "gene segment," or "segment" includes reference to a V (light or heavy) or D or J (light or heavy) immunoglobulin gene segment, which includes unrearranged sequences at immunoglobulin loci (in *e.g.*, humans and mice) that can participate in a rearrangement (mediated by, *e.g.,* endogenous recombinases) to form a rearranged V/J or V/D/J sequence. Unless indicated otherwise, the V, D, and J segments comprise recombination signal sequences (RSS) that allow for V/J recombination or V/D/J recombination according to the 12/23 rule. Unless indicated otherwise, the segments further comprise sequences with which they are associated in nature or functional equivalents thereof (*e.g.*, for V segments promoter(s) and leader(s)).

[00381] The phrase "heavy chain," or "immunoglobulin heavy chain" includes an immunoglobulin heavy chain constant region sequence from any organism, and unless otherwise specified includes a heavy chain variable domain (V_H). V_H domains include three heavy chain CDRs and four framework (FR) regions, unless otherwise specified. Fragments of heavy chains include CDRs, CDRs and FRs, and combinations thereof. A typical heavy chain consists essentially of, following the variable domain (from N-terminal to C-terminal), a C_H1 domain, a hinge, a C_H2 domain, a C_H3 domain, and optionally a C_H4 domain (*e.g.*, in the case of IgM or IgE) and a transmembrane (M) domain (*e.g.*, in the case of membrane-bound immunoglobulin on lymphocytes). A heavy chain constant region is a region of a heavy chain that extends (from N-terminal side to C-terminal side) from outside FR4 to the C-terminal of the heavy chain. Heavy chain constant regions with minor deviations, *e.g.,* truncations of one, two, three or several amino acids from the C-terminal, would be encompassed by the phrase "heavy chain constant region," as well as heavy chain constant regions with sequence modifications, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions. Amino acid substitutions can be made at one or more positions selected from, *e.g.* (with reference to EU numbering of an immunoglobulin constant region, *e.g.*, a human IgG constant region), 228, 233, 234, 235, 236, 237, 238, 239, 241, 248, 249, 250, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292,

293, 294, 295, 296, 297, 298, 301, 303, 305, 307, 308, 309, 311, 312, 315, 318, 320, 322, 324, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 337, 338, 339, 340, 342, 344, 356, 358, 359, 360, 361, 362, 373, 375, 376, 378, 380, 382, 383, 384, 386, 388, 389, 398, 414, 416, 419, 428, 430, 433, 434, 435, 437, 438, and 439.

[00382] For example, and not by way of limitation, a heavy chain constant region can be modified to exhibit enhanced serum half-life (as compared with the same heavy chain constant region without the recited modification(s)) and have a modification at position 250 (*e.g.,* E or Q); 250 and 428 (*e.g.,* L or F); 252 (*e.g.,* L/Y/F/W or T), 254 (*e.g.,* S or T), and 256 (*e.g.,* S/R/Q/E/D or T); or a modification at 428 and/or 433 (*e.g.,* L/R/SI/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at 250 and/or 428; or a modification at 307 or 308 (*e.g.,* 308F, V308F), and 434. In another example, the modification can comprise a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and a 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); a 307 and/or 308 modification (*e.g.,* 308F or 308P).

[00383] The phrase "light chain" includes an immunoglobulin light chain constant (C_L) region sequence from any organism, and unless otherwise specified includes human κ and λ light chains. Light chain variable (V_L) domains typically include three light chain CDRs and four framework (FR) regions, unless otherwise specified. Generally, a full-length light chain (V_L + C_L) includes, from amino terminus to carboxyl terminus, a V_L domain that includes FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, and a C_L region. Light chains (V_L + C_L) that can be used with this invention include those, *e.g.,* that do not selectively bind either a first or second (in the case of bispecific binding proteins) epitope selectively bound by the binding protein (*e.g.,* the epitope(s) selectively bound by the V_L domain fused with the C_H domain). V_L domains that do not selectively bind the epitope(s) bound by the V_L that is fused with the C_H domain include those that can be identified by screening for the most commonly employed light chains in existing antibody libraries (wet libraries or *in silico*), wherein the light chains do not substantially interfere with the affinity and/or selectivity of the epitope binding domains of the binding proteins. Suitable light chains include those that can bind (alone or in combination with its cognate V_L fused with the C_H region) an epitope that is specifically bound by the V_L fused to the C_H region.

[00384] The phrase "micromolar range" is intended to mean 1-999 micromolar; the phrase "nanomolar range" is intended to mean 1-999 nanomolar; the phrase "picomolar range" is intended to mean 1-999 picomolar.

[00385] The term "non-human animals" is intended to include any non-human animals such as cyclostomes, bony fish, cartilaginous fish such as sharks and rays, amphibians,

reptiles, mammals, and birds. Suitable non-human animals include mammals. Suitable mammals include non-human primates, goats, sheep, pigs, dogs, cows, and rodents. Suitable non-human animals are selected from the rodent family including rat and mouse. In one embodiment, the non-human animals are mice.

[00386] 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, incorporated herein by reference). 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.

[00387] 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, N. (2005), Human antibodies from transgenic animals. *Nat Biotechnol* 23:1117-1125; 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 endogenous 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; 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, *N 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: (1) 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* 176I6839-6851); (2) 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 (3) 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. Further such reverse chimeric antibodies may be readily reformatted into fully human antibodies for therapeutic purposes.

[00388] 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 sequences are derived can vary widely. Exemplary heterologous immunoglobulin sequences include human sequences.

[00389] 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).

[00390] 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.

[00391] 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 one or more human V_L and one or more human J_L gene segments, wherein the one or more human V_L and one or more J_L 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 one or more human V_L gene segments include human $V\kappa$ or human $V\lambda$ gene segments. In one embodiment, the one or more human J_L gene segments include human $J\kappa$ or human $J\lambda$ gene segments.

[00392] A method for a large *in situ* genetic replacement of the mouse germline immunoglobulin heavy chain variable genes with human germline immunoglobulin light chain variable genes while maintaining the ability of the mice to generate offspring is described. Specifically, the precise replacement of the mouse heavy chain variable gene loci with human light chain variable gene loci while leaving the mouse constant regions intact is described. As a result, mice have been created that express immunoglobulin-like binding proteins in the context of endogenous constant regions. The human light chain variable regions are linked to mouse heavy chain constant regions to form chimeric human-mouse immunoglobulin loci that rearrange and express unique immunoglobulin-like molecules. The

immunoglobulin-like molecules expressed are "reverse chimeras," *i.e.*, they comprise human variable region sequences and mouse constant region sequences.

[00393] 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.

[00394] Notwithstanding the near wild-type humoral immune function observed in mice with replaced immunoglobulin loci, there are other challenges encountered when employing a direct replacement of the immunoglobulin 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 heavy chain locus are optimally present in mice with replaced immunoglobulin loci, due to their role in fertility.

[00395] 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 human immunoglobulin light chain variable gene loci (V_L-J_L) is 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. 2 – FIG. 6). Engineering steps 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 were performed (FIG. 7 – FIG. 9). Specifically, about half a megabase of the human immunoglobulin κ light chain locus containing the proximal arm

(*i.e.,* 40 functional human Vκ gene segments and five human Jκ 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).

Genomic Location and Function of Mouse ADAM6

[00396] Male mice that lack the ability to express any functional ADAM6 protein exhibit a severe 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 heavy chain variable gene segments with human light chain variable gene segments. The loss of ADAM6 function results because the ADAM6 locus is located within a region of the endogenous immunoglobulin heavy chain variable 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 heavy chain variable gene segments with human light chain variable gene segments, it is generally a cumbersome approach to set up males and females that are each homozygous for the replacement and await a productive mating. Successful litters are relatively rare, and average litter size is very low. Instead, males heterozygous for the replacement have been employed to mate with females homozygous for the replacement to generate progeny that are heterozygous for the replacement, then breed a homozygous mouse 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.

[00397] 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 modifications to an endogenous immunoglobulin heavy chain locus, various modified breeding or propagation schemes are employed. The low fertility, or infertility, of male mice homozygous for a replacement of the endogenous 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 a replacement.

[00398] 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.

[00399] 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.

[00400] 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.

[00401] 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.

[00402] 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.

[00403] 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.

[00404] 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.

[00405] 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.

[00406] 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 light chain sequences, and optionally one or more human D_H gene segments, 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.

[00407] The ADAM6 protein is a member of the A Disintegrin And Metalloprotease (ADAM) family of proteins, which is a large family with 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.

[00408] In humans, an ADAM6 gene, reportedly a pseudogene, is located between human V_H gene segments V_H1-2 and V_H6-1. In mice, there are two ADAM6 genes—ADAM6a and ADAM6b—that are located in an intergenic region between mouse V_H and D_H gene segments, and 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 damaged endogenous ADAM6 loci.

[00409] The position of the intergenic sequence in mice that encodes ADAM6a and ADAM6b renders the intergenic sequence susceptible to modification when modifying an endogenous 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 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. In various embodiments, the complementing nucleotide sequence encodes a mouse ADAM6a protein as set forth in SEQ ID NO: 1, and/or encodes a mouse ADAM6b protein as set forth in SEQ ID NO: 2. 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.

[0001] The nucleotide sequence that rescues fertility can be placed at any suitable position. It can be placed in an intergenic region (*e.g.,* between V and J gene segments or upstream of V gene segments), 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, herein incorporated by reference.

[0002] 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).

[0003] 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.

[0004] 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.

[00410] 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 genome of the mouse than in a willd-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.

[00411] 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 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 be 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.

[00412] Another example of an ectopic placement is placement within a modified immunoglobulin heavy chain locus. For example, a mouse comprising a replacement of one or more endogenous V_H gene segments with human V_L gene segments, wherein the replacement removes an endogenous ADAM6 sequence, can be engineered to have a mouse ADAM6 sequence located within sequence that contains the human V_L gene segments. The resulting modification would generate a (ectopic) mouse ADAM6 sequence within a human gene sequence, and the (ectopic) placement of the mouse ADAM6 sequence within the human gene sequence can approximate the position of the human ADAM6 pseudogene (*i.e.*, between two V segments) or 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 light chain 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.

[00413] 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.

[00414] 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).

[00415] 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.

[00416] 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.

[00417] 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.

[00418] 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)).

[00419] 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.

[00420] 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.

[00421] 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. In a specific embodiment, the mouse ADAM6a comprises SEQ ID NO: 1 or a functional fragment thereof, and the mouse ADAM6b comprises SEQ ID NO: 2 or a functional fragment thereof.

[00422] In one aspect, non-human animals are provided, wherein the non-human animals comprise (a) an insertion of one or more human V_L and J_L gene segments upstream of a non-human immunoglobulin heavy chain constant region, (b) an insertion of one or more human V_L, and J_L gene segments upstream of a non-human immunoglobulin light chain constant region, and (c) a nucleotide sequence that encodes an ADAM6 protein or a

functional fragment thereof. In one embodiment, the non-human heavy and/or light chain constant regions are rodent constant regions (*e.g.*, selected from mouse, rat or hamster constant regions). In one embodiment, the non-human light chain constant region is a rodent constant region. In a specific embodiment, the light chain constant region is a mouse Cκ or a rat Cκ region. In a specific embodiment, the light chain constant region is a mouse Cλ or a rat Cκ region. In one embodiment, the human V_L and J_L gene segments are Vκ and Jκ gene segments. In one embodiment, the human V_L and J_L gene segments are Vλ and Jλ gene segments. In one embodiment, the non-human animal further comprises one or more human D_H gene segments present between the human V_L and J_L gene segments. Suitable non-human animals include rodents, *e.g,* mice, rats and hamsters. In one embodiment, the rodent is a mouse or a rat.

[00423] In one embodiment, the non-human animal comprises at least six to at least 40 human Vκ gene segments and at least one to at least five human Jκ gene segments. In a specific embodiment, the non-human animal comprises six human Vκ gene segments and five human Jκ gene segments. In a specific embodiment, the non-human animal comprises 16 human Vκ gene segments and five human Jκ gene segments. In a specific embodiment, the non-human animal comprises 30 human Vκ gene segments and five human Jκ gene segments. In a specific embodiment, the non-human animal comprises 40 human Vκ gene segments and five human Jκ gene segments. In various embodiments, the human Jκ gene segments are selected from Jκ1, Jκ2, Jκ3, Jκ4, Jκ5, and a combination thereof.

[00424] In one embodiment, the nucleotide sequence that encodes an ADAM6 protein or functional fragment thereof is ectopic in the non-human animal. In one embodiment, the nucleotide sequence that encodes an ADAM6 protein or functional fragment thereof (that is functional in the non-human animal) is present the same location as compared to a wild-type type non-human ADAM6 locus. In one embodiment, the non-human animal is a mouse and the nucleotide sequence encodes a mouse ADAM6 protein or functional fragment thereof and is present at an ectopic location in the genome of the non-human animal. In one embodiment the non-human animal is a mouse and the nucleotide sequence encodes a mouse ADAM6 protein or functional fragment thereof and is present within immunoglobulin gene segments. In a specific embodiment, the immunoglobulin gene segments are heavy chain gene segments of the non-human animal. In a specific embodiment, the immunoglobulin gene segments are light chain gene segments of another species. In one embodiment, the light chain gene segments are human κ light chain gene segments. In one embodiment, the mouse comprises an ectopic contiguous sequence comprising one or more

endogenous unrearranged heavy chain gene segments, and the ADAM6 sequence is within the ectopic contiguous sequence.

[00425] In one embodiment, the non-human animal lacks an endogenous immunoglobulin V_L and/or a J_L gene segment at an endogenous immunoglobulin light chain locus. In one embodiment, the non-human animal comprises endogenous immunoglobulin V_L and/or J_L gene segments that are incapable of rearranging to form an immunoglobulin V_L domain in the non-human animal. In one embodiment, all or substantially all endogenous immunoglobulin Vκ and Jκ gene segments are replaced with one or more human Vκ and Jκ gene segments. In one embodiment, all or substantially all endogenous immunoglobulin Vλ and Jλ gene segments are deleted in whole or in part. In one embodiment, all or substantially all endogenous immunoglobulin V_L and J_L gene segments are intact in the non-human animal and the non-human animal comprises one or more human Vκ gene segments and one or more human Jκ gene segments inserted between endogenous immunoglobulin V_L and/or J_L gene segments and an endogenous immunoglobulin light chain constant region. In a specific embodiment, the intact endogenous immunoglobulin V_L and J_L gene segments are rendered incapable of rearranging to form a V_L domain of an antibody in the non-human animal. In one embodiment, the endogenous immunoglobulin light chain locus of the non-human animal is an immunoglobulin κ light chain locus. In one embodiment, the endogenous immunoglobulin V_L and J_L gene segments are Vκ and Jκ gene segments.

[00426] In one aspect, cells and/or tissues derived from non-human animals as described herein are provided, wherein the cells and/or tissues comprise (a) an insertion of one or more human Vκ and Jκ gene segments upstream of an non-human immunoglobulin light chain constant region, (b) an insertion of one or more human Vκ and Jκ gene segments upstream of an non-human immunoglobulin heavy chain constant region, and (c) a nucleotide sequence that encodes an ADAM6 protein or a functional fragment thereof. In one embodiment, the non-human heavy and/or light chain constant regions are mouse constant regions. In one embodiment, the non-human heavy and/or light chain constant regions are rat constant regions. In one embodiment, the non-human heavy and/or light chain constant regions are hamster constant regions.

[00427] In one embodiment, the nucleotide sequence that encodes an ADAM6 protein or functional fragment thereof is ectopic in the cell and/or tissue. In one embodiment, the nucleotide sequence that encodes an ADAM6 protein or functional fragment thereof is present the same location as compared to a wild-type type non-human ADAM6 locus. In one embodiment the non-human cell and/or tissue is derived from a mouse and the nucleotide sequence encodes a mouse ADAM6 protein or functional fragment thereof and is

present at an ectopic location. In one embodiment, the non-human cell and/or tissue is derived from a mouse and the nucleotide sequence encodes a mouse ADAM6 protein or functional fragment thereof and is present within immunoglobulin gene segments. In a specific embodiment, the immunoglobulin gene segments are heavy chain gene segments. In a specific embodiment, the immunoglobulin gene segments are light chain gene segments. In one embodiment, a contiguous sequence of endogenous heavy chain gene segments are placed ectopically in the non-human animal, wherein the contiguous sequence of ectopically placed endogenous heavy chain gene segments comprises an ADAM6 gene that is functional in the mouse (*e.g.*, in a male mouse).

[00428] In one aspect, use of a non-human animal as described herein to make an antigen-binding protein is provided, wherein the non-human animal expresses (a) an antibody that comprises (i) an immunoglobulin light chain that comprises a human Vκ domain and a non-human light chain constant region and (ii) an immunoglobulin heavy chain that comprises a human Vκ domain and a non-human constant region; and (b) an ADAM6 protein or functional fragment thereof. In one embodiment, the antigen binding protein is human. In one embodiment, the non-human animal is a rodent and the non-human constant regions are rodent constant regions. In a specific embodiment, the rodent is a mouse.

[00429] In one aspect, a non-human cell or tissue derived from a non-human animal as described herein is provided. In one embodiment, the non-human cell or tissue comprises one or more human immunoglobulin Vκ gene segments and at least one human immunoglobulin Jκ gene segments contiguous with a non-human immunoglobulin light chain constant region gene and one or more human Vκ and one or more human Jκ gene segments contiguous with a non-human immunoglobulin heavy chain constant region gene, wherein the cell or tissue expresses an ADAM6 protein or functional fragment thereof. In one embodiment, the non-human light chain constant region gene is a mouse Cκ.

[00430] In one embodiment, the nucleotide sequence that encodes the ADAM6 protein or functional fragment thereof is ectopic. In one embodiment, the nucleotide sequence that encodes the ADAM6 protein or functional fragment thereof is located at a position that is the same as a wild-type non-human cell. In various embodiments, the non-human cell is a mouse B cell. In various embodiments, the non-human cell is an embryonic stem cell.

[00431] In one embodiment, the tissue is derived from spleen, bone marrow or lymph node of the non-human animal.

[00432] In one aspect, use of a cell or tissue derived from a non-human animal as described herein to make a hybridoma or quadroma is provided.

[00433] In one aspect, a non-human cell comprising a modified genome as described herein is provided, wherein the non-human cell is an oocyte, a host embryo, or a fusion of a cell from a non-human animal as described herein and a cell from a different non-human animal.

[00434] In one aspect, use of a cell or tissue derived from a non-human animal as described herein to make a human antigen-binding protein is provided. In one embodiment, the human antigen-binding protein comprises a human $V\kappa$ domain isolated from a non-human animal as described herein.

[00435] In one aspect, a method for making an antigen-binding protein that binds to an antigen of interest is provided, wherein the method comprises (a) exposing a non-human animal as described herein to an antigen of interest, (b) isolating one or more B lymphocytes of the non-human animal, wherein the one or more B lymphocytes express a V_L binding protein that binds the antigen of interest, and (c) identifying a nucleic acid sequence that encodes a V_L domain of the V_L binding protein that binds the antigen of interest, wherein the V_L binding protein comprises a human $V\kappa$ domain and a non-human light chain constant domain and a human $V\kappa$ domain and a non-human heavy chain constant domain, and (d) employing the nucleic acid sequence of (c) with a human immunoglobulin constant region nucleic acid sequence to make a human antigen-binding protein that binds the antigen of interest.

[00436] In one embodiment, the non-human light chain constant domain of the V_L binding protein is a mouse $C\kappa$. In one embodiment, the non-human heavy chain chain constant domain of the V_L binding protein is a mouse $C\gamma$. In one embodiment, the non-human animal is a mouse.

[00437] In one aspect, a fertile male mouse comprising a modification at an immunoglobulin heavy chain locus is provided, wherein the fertile male mouse comprises an ectopic ADAM6 sequence that is functional in the male mouse.

Ectopic ADAM6 in Modified Immunoglobulin Heavy Chain Loci

[00438] 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.

[00439] Mice that make human antibodies (*i.e.,* human variable regions) 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.

[00440] 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.

[00441] 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.

[00442] 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.

[00443] A primary concern in making antibody-based human therapeutics is making a sufficiently large diversity of human immunoglobulin variable region sequences to identify useful variable domains that specifically recognize particular epitopes and bind them with a

desirable affinity, usually—but not always—with high affinity. Prior to the development of VELOCIMMUNE® mice (described herein), there was no indication that mice expressing human variable regions with mouse constant regions would exhibit any significant differences from mice that made human antibodies from a transgene. That supposition, however, was incorrect.

[00444] VELOCIMMUNE® mice, which contain a precise replacement of mouse immunoglobulin variable regions with human immunoglobulin variable regions at the endogenous loci, display a surprising and remarkable similarity to wild-type mice with respect to B cell development. In a surprising and stunning development, VELOCIMMUNE® mice displayed an essentially normal, wild-type response to immunization that differed only in one significant respect from wild-type mice—the variable regions generated in response to immunization are fully human.

[00445] VELOCIMMUNE® mice contain a precise, large-scale replacement of germline variable regions of mouse immunoglobulin heavy chain (IgH) and immunoglobulin light chain (e.g., κ light chain, Igκ) with corresponding human immunoglobulin variable regions, at the endogenous loci. In total, about six megabases of mouse loci are replaced with about 1.5 megabases of human genomic sequence. This precise replacement results in a mouse with hybrid immunoglobulin loci that make heavy and light chains that have a human variable regions and a mouse constant region. The precise replacement of mouse V_H-D_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 rich diversity of human variable regions is generated in the mouse upon antigen challenge.

[00446] VELOCIMMUNE® mice are possible because immunoglobulin gene segments for heavy and κ light chains rearrange similarly in humans and mice, which is not to say that their loci are the same or even nearly so—clearly they are not. However, the loci are similar enough that humanization of the heavy chain variable gene locus can be accomplished by replacing about three million base pairs of contiguous mouse sequence that contains all the V_H, D_H, and J_H gene segments with about one million bases of contiguous human genomic sequence covering basically the equivalent sequence from a human immunoglobulin locus.

[00447] 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, 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 variable gene segment usage.

[00448] 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.

[00449] Large-scale humanization by recombineering methods were used to modify mouse embryonic stem (ES) cells to create a unique immunoglobulin heavy chain locus by precisely replacing up to three megabases of the mouse heavy chain immunoglobulin locus including essentially all of the mouse V_H, D_H, and J_H gene segments with 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. Additionally, 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. Mice with such replaced immunoglobulin loci can comprise a disruption or deletion of the 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 mouse ADAM6 locus.

[00450] Mice are described that comprise the replaced 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 is placed between a human V_L gene segment and a human J_L gene segment or upstream of a 5'-most human V_L gene segment at the modified endogenous heavy chain locus. The direction of transcription of the ADAM6 genes may be opposite (FIG. 7) or the same (FIG. 8) with respect to the direction of transcription of the surrounding human V_L gene segments. Although examples herein show rescue of fertility by placing the ectopic sequence between

the indicated human V_L and J_L gene segments or upstream of a 5'-most human V_L gene segment, skilled persons will recognize that placement of the ectopic sequence at any suitable transcriptionally-permissive locus in the mouse genome (or even extra-chromosomally) will be expected to similarly rescue fertility in a male mouse. In various embodiments, the ectopic nucleic acid sequence is selected from SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5, wherein the ectopic sequence encodes one or more ADAM6 proteins, wherein the one or more ADAM6 proteins comprise SEQ ID NO: 1, SEQ ID NO: 2 or a combination thereof.

[00451] 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.

[00452] 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 segments and J_H gene segments with human J_L 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_L – mouse C_H1 – mouse C_H2 – mouse C_H3; (b) human V_L –mouse hinge – mouse C_H2 – mouse C_H3; and, (c) human V_L – mouse C_H2 – mouse C_H3.

[00453] In one aspect, the nucleotide sequence that rescues fertility is placed within a human immunoglobulin light chain variable region sequence (*e.g.*, between human Vκ4-1 and Jκ1 gene segments) in a mouse that has a replacement of all or substantially all mouse immunoglobulin heavy chain variable gene segments (mV_H's, mD_H's, and mJ_H's) with one or more human immunoglobulin κ light chain variable gene segments (hVκ's and hJκ's), and the mouse further comprises a replacement of all or substantially all mouse immunoglobulin κ light chain variable gene segments (mVκ's, mJκ's) with one or more human immunoglobulin κ light chain variable gene segments (hVκ's and hJκ's).

[00454] In one aspect, a functional mouse ADAM6 locus (or ortholog or homolog or functional fragment thereof) can be placed in the midst of human V_L gene segments or upstream of a 5'-most human V_L gene segment, wherein the human V_L gene segments replace endogenous V_H gene segments. In one embodiment, all or substantially all mouse V_H gene segments are removed and replaced with one or more human V_L gene segments, and the mouse ADAM6 locus is placed immediately adjacent to the 5' end of the 5'-most human V_L gene segments, or between two human V_L gene segments. In a specific embodiment, the mouse ADAM6 locus is placed between two V_L gene segments near the 3' terminus of the inserted human 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 V_L gene segments): human Vκ5-2–mouse ADAM6 locus–human Vκ4-1. 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 V_L gene segments): mouse ADAM6 locus–human Vκ2-40, wherein human Vκ2-40 is the 5'-most human V_L gene segment at the modified immunoglobulin heavy chain locus. 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 V_L gene segments. In one embodiment, the orientation of one or more of mouse ADAM6a and mouse ADAM6b of the mouse ADAM6 locus is the same with respect to direction of transcription as compared with the orientation of the human V_L gene segments.

[00455] In one aspect, a functional mouse ADAM6 locus (or ortholog or homolog or functional fragment thereof) can be placed between a human V_L gene segment and a human J_L gene segment (*i.e.,* in the intergenic region between the 3'-most human V_L gene segment and the 5'-most J_L gene segment), wherein the human V_L and J_L gene segments replace endogenous V_H gene segments. In one embodiment, all or substantially all mouse V_H gene segments are removed and replaced with one or more human V_L gene segments and one or more human J_L gene segments, and the mouse ADAM6 locus is placed immediately adjacent to the 3' end of the 3'-most human V_L gene segment and immediately adjacent to the 5' end of the 5'-most human J_L gene segment. In a specific embodiment, the one or more human V_L gene segments and one or more human J_L gene segments are Vκ and Jκ 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 V_L gene segments): human Vκ4-1–mouse ADAM6 locus–human Jκ1. 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 V_L gene segments. In one embodiment, the orientation of one or more of mouse ADAM6a and mouse ADAM6b of the mouse ADAM6 locus is the same with respect to direction of transcription as compared with the orientation of the human V_L gene segments.

[00456] A mouse modified with one or more human V_L gene segments (*e.g.,* $V\kappa$ or $V\lambda$ segments) replacing all or substantially all endogenous V_H gene segments can be modified so as to either maintain the endogenous 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.,* $V\lambda$ + m/hD_H), or a J gene segment (whether human or mouse, *e.g.,* $V\kappa$ + 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 placed 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 V_L gene segments. Alternatively, the mouse ADAM6 locus can be placed in the intergenic region between the 3'-most human V_L gene segment and the 5'-most J_L gene segment.

[00457] In one aspect, a mouse is provided with a replacement of one or more endogenous V_H gene segments, and that comprises at least one endogenous D_H gene segment. In such a mouse, the modification of the endogenous 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 ADAM6 locus nonfunctional. For example, in one embodiment the mouse comprises a replacement of all or substantially all endogenous V_H gene segments with one or more human V_L gene segments, and the mouse comprises one or more endogenous D_H gene segments and a functional endogenous ADAM6 locus.

[00458] In another embodiment, the mouse comprises the modification of endogenous 3'-most V_H gene segments, and a modification of one or more endogenous D_H gene segments, and the modification is carried out so as to maintain the integrity of the endogenous 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 V_H gene segments with one or more human V_L 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 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.

[00459] 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.

[00460] 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 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 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.

[00461] 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.

[00462] 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; a replacement of all or substantially all mouse D_H and J_H gene segments with one or more human J_L gene segments.

[00463] 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.

[00464] 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.

[00465] 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 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.

[00466] 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.

[00467] 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.

[00468] 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.

[00469] 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.

[00470] 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.

[00471] 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.

[00472] 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.

[00473] In one aspect, a non-human animal is provided, comprising a modified

immunoglobulin heavy chain locus, wherein the modified immunoglobulin heavy chain locus comprises a non-human ADAM6 sequence or ortholog or homolog thereof.

[00474] In one embodiment, the non-human animal is a rodent selected from a mouse, a rat, and a hamster.

[00475] 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.

[00476] 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.

[00477] 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.

[00478] In one embodiment, the modified immunoglobulin heavy chain locus comprises one or more human V_L gene segments and one or more human J_L gene segments. In a specific embodiment, the one or more human V_L gene segments and one or more human J_λ 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. In one embodiment, the human VL and JL gene segments are human $V\kappa$ and $J\kappa$ gene segments.

[00479] In one embodiment, the non-human ADAM6 sequence is contiguous with a human immunoglobulin light chain sequence. In one embodiment, the non-human ADAM6 sequence is positioned within a human immunoglobulin light chain sequence. In a specific embodiment, the human immunoglobulin light chain sequence comprises a V and/or J gene

segment.

[00480] 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 J gene segment. In one embodiment, the mouse ADAM6 sequence is juxtaposed between two J gene segments.

[00481] In one aspect, a genetically modified non-human animal is provided, comprising a B cell that expresses a human V_L 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.

[00482] 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.

[00483] 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.

[00484] In one embodiment, the human 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.

[00485] In various embodiments, the human V_L domains are human $V\kappa$ domains.

[00486] 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.

[00487] 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.

[00488] In one embodiment, the human immunoglobulin sequence is an immunoglobulin light chain sequence. In one embodiment, the immunoglobulin sequence comprises one or more V_L gene segments. In one embodiment, the human immunoglobulin sequence comprises one or more J_L gene segments. In one embodiment, the human immunoglobulin sequence comprises one or more V_L gene segments and one or more J_L gene segments. In various embodiments, the human V_L and J_L gene segments are human $V\kappa$ and $J\kappa$ gene segments.

[00489] In one aspect, a mouse is provided with a disabled endogenous immunoglobulin heavy chain locus, comprising a disabled or deleted endogenous 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.

[00490] In one aspect, a mouse is provided with a disabled endogenous immunoglobulin heavy chain locus, comprising a functional endogenous 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 at the endogenous immunoglobulin heavy chain locus at a position upstream from one or more endogenous heavy chain constant region genes. 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.

Bispecific-Binding Proteins

[00491] The binding proteins described herein, and nucleotide sequences encoding them, can be used to make multispecific binding proteins, *e.g.*, bispecific binding proteins. In this aspect, a first polypeptide consisting essentially of a first V_L domain fused with a C_H region can associate with a second polypeptide consisting essentially of a second V_L domain fused with a C_H region. Where the first V_L domain and the second V_L domain specifically bind a different epitope, a bispecific-binding molecule can be made using the two V_L domains. The

C_H region can be the same or different. In one embodiment, *e.g.*, one of the C_H regions can be modified so as to eliminate a protein A binding determinant, whereas the other heavy chain constant region is not so modified. This particular arrangement simplifies isolation of the bispecific binding protein from, *e.g.*, a mixture of homodimers (*e.g.*, homodimers of the first or the second polypeptides).

[00492] In one aspect, the methods and compositions described herein are used to make bispecific-binding proteins. In this aspect, a first V_L that is fused to a C_H region and a second V_L that is fused to a C_H region are each independently cloned in frame with a human IgG sequence of the same isotype (*e.g.*, a human IgG1, IgG2, IgG3, or IgG4). The first V_L specifically binds a first epitope, and the second V_L specifically binds a second epitope. The first and second epitopes may be on different antigens, or on the same antigen.

[00493] In one embodiment, the IgG isotype of the C_H region fused to the first V_L and the IgG isotype of the C_H region fused to the second V_L are the same isotype, but differ in that one IgG isotype comprises at least one amino acid substitution. In one embodiment, the at least one amino acid substitution renders the heavy chain bearing the substitution unable or substantially unable to bind protein A as compared with the heavy chain that lacks the substitution.

[00494] In one embodiment, the first C_H region comprises a first C_H3 domain of a human IgG selected from IgG1, IgG2, and IgG4; and the second C_H region comprises a second C_H3 domain of a human IgG selected from IgG1, IgG2, and IgG4, wherein the second C_H3 domain comprises a modification that reduces or eliminates binding of the second C_H3 domain to protein A.

[00495] In one embodiment, the second C_H3 domain comprises a 435R modification, numbered according to the EU index of Kabat. In another embodiment, the second C_H3 domain further comprises a 436F modification, numbered according to the EU index of Kabat.

[00496] In one embodiment, the second C_H3 domain is that of a human IgG1 that comprises a modification selected from the group consisting of D356E, L358M, N384S, K392N, V397M, and V422I, numbered according to the EU index of Kabat.

[00497] In one embodiment, the second C_H3 domain is that of a human IgG2 that comprises a modification selected from the group consisting of N384S, K392N, and V422I, numbered according to the EU index of Kabat.

[00498] In one embodiment, the second C_H3 domain is that of a human IgG4 comprising a modification selected from the group consisting of Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I, numbered according to the EU index of Kabat.

[00499] In one embodiment, the binding protein comprises C_H regions having one or more modifications as recited herein, wherein the constant region of the binding protein is nonimmunogenic or substantially nonimmunogenic in a human. In a specific embodiment, the C_H regions comprise amino acid sequences that do not present an immunogenic epitope in a human. In another specific embodiment, the binding protein comprises a C_H region that is not found in a wild-type human heavy chain, and the C_H region does not comprise a sequence that generates a T-cell epitope.

EXAMPLES

[00500] The following examples are provided so as to describe 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. Introduction of Human Light Chain Gene Segments Into A Heavy Chain Locus

[00501] Various targeting constructs were made 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) to modify mouse genomic Bacterial Artificial Chromosome (BAC) libraries. Mouse BAC DNA was modified by homologous recombination to inactivate the endogenous heavy chain locus through targeted deletion of V_H, D_H and J_H gene segments for the ensuing insertion of unrearranged human germline κ light chain gene sequences (*e.g.,* see top of FIG. 2).

[00502] Briefly, the mouse heavy chain locus was deleted in two successive targeting events using recombinase-mediated recombination. The first targeting event included a targeting at the 5' end of the mouse heavy chain locus using a targeting vector comprising from 5' to 3' a 5' mouse homology arm, a recombinase recognition site, a neomycin cassette and a 3' homology arm. The 5' and 3' homology arms contained sequence 5' of the mouse heavy chain locus. The second targeting event included a targeting at the 3' end of the mouse heavy chain locus in the region of the J_H gene segments using a second targeting vector that contained from 5' to 3' a 5' mouse homology arm, a 5' recombinase recognition site, a second recombinase recognition site, a hygromycin cassette, a third recombinase recognition site, and a 3' mouse homology arm. The 5' and 3' homology arms contained sequence flanking the mouse J_H gene segments and 5' of the intronic enhancer and constant regions. Positive ES cells containing a modified heavy chain locus targeted with

both targeting vectors (as described above) were confirmed by karyotyping. DNA was then isolated from the double-targeted ES cells and subjected to treatment with a recombinase thereby mediating the deletion of genomic DNA of the mouse heavy chain locus between the 5' recombinase recognition site in the first targeting vector and the 5' recombinase recognition site in the second targeting vector, leaving a single recombinase recognition site and the hygromycin cassette flanked by two recombinase recognition sites (top of FIG. 2). Thus a modified mouse heavy chain locus containing intact C_H genes was created for progressively inserting human κ germline gene segments in a precise manner using targeting vectors described below.

[00503] Four separate targeting vectors were engineered to progressively insert 40 human Vκ gene segments and five human Jκ gene segments into the inactivated mouse heavy chain locus (described above) using standard molecular techniques recognized in the art (FIG. 2). The human κ gene segments used for engineering the four targeting constructs are naturally found in proximal contig of the germline human κ light chain locus (bottom of FIG. 1 and Table 1).

[00504] A ~110,499 bp human genomic fragment containing the first six human Vκ gene segments and five human Jκ gene segments was engineered to contain a PI-SceI site 431 bp downstream (3') of the human Jκ5 gene segment. Another PI-SceI site was engineered at the 5' end of a ~7,852 bp genomic fragment containing the mouse heavy chain intronic enhancer, the IgM switch region (Sμ) and the IgM gene of the mouse heavy chain locus. This mouse fragment was used as a 3' homology arm by ligation to the ~110.5 kb human fragment, which created a 3' junction containing, from 5' to 3', ~110.5 kb of genomic sequence of the human κ light chain locus containing the first six consecutive Vκ gene segments and five Jκ gene segments, a PI-SceI site, ~7,852 bp of mouse heavy chain sequence containing the mouse intronic enhancer, Sμ and the mouse IgM constant gene. Upstream (5') from the human Vκ1-6 gene segment was an additional 3,710 bp of human κ sequence before the start of the 5' mouse homology arm, which contained 19,752 bp of mouse genomic DNA corresponding to sequence 5' of the mouse heavy chain locus. Between the 5' homology arm and the beginning of the human κ sequence was a neomycin cassette flanked by three recombinase recognition sites (see Targeting Vector 1, FIG. 2). The final targeting vector for the first insertion of human κ sequence from 5' to 3' included a 5' homology arm containing ~20 kb of mouse genomic sequence 5' of the heavy chain locus, a first recombinase recognition site (R1), a neomycin cassette, a second recombinase recognition site (R2), a third recombinase recognition site (R3), ~110.5 kb of human genomic κ sequence containing the first six consecutive human Vκ gene segments and five

human Jκ gene segments, a PI-SceI site, and a 3' homology arm containing ~8 kb of mouse genomic sequence including the intronic enhancer, Sμ and the mouse IgM constant gene (FIG. 2, Targeting Vector 1). Homologous recombination with this targeting vector created a modified mouse heavy chain locus containing six human Vκ gene segments and five human Jκ gene segments operably linked to the endogenous heavy chain constant genes which, upon recombination, leads to the formation of a hybrid heavy chain (*i.e.*, a human Vκ domain and a mouse C_H region).

TABLE 1

Targeting Vector	Size of Human κ Sequence	Human κ Gene Segments Added	
		Vκ	Jκ
1	~110.5 kb	4-1, 5-2, 7-3, 2-4, 1-5, 1-6	1 – 5
2	~140 kb	3-7, 1-8, 1-9, 2-10, 3-11, 1-12, 1-13, 2-14, 3-15, 1-16	-
3	~161 kb	1-17, 2-18, 2-19, 3-20, 6-21, 1-22, 1-23, 2-24, 3-25, 2-26, 1-27, 2-28, 2-29, 2-30	-
4	~90 kb	3-31, 1-32, 1-33, 3-34, 1-35, 2-36, 1-37, 2-38, 1-39, 2-40	-

[00505] Introduction of ten additional human Vκ gene segments into a hybrid heavy chain locus. A second targeting vector was engineered for introduction of 10 additional human Vκ gene segments to the modified mouse heavy chain locus described above (see FIG. 2, Targeting Vector 2). A 140,058 bp human genomic fragment containing 12 consecutive human Vκ gene segments from the human κ light chain locus was engineered with a 5' homology arm containing mouse genomic sequence 5' of the mouse heavy chain locus and a 3' homology arm containing human genomic κ sequence. Upstream (5') from the human Vκ1-16 gene segment was an additional 10,170 bp of human κ sequence before the start of the 5' mouse homology arm, which was the same 5' homology arm used for construction of Targeting Vector 1 (FIG. 2). Between the 5' homology arm and the beginning of the human κ sequence was a hygromycin cassette flanked by recombinase recognition sites. The 3' homology arm included a 31,165 bp overlap of human genomic κ sequence corresponding to the equivalent 5' end of the ~110.5 kb fragment of human genomic κ sequence of Targeting Vector 1 (FIG. 2). The final targeting vector for the insertion of 10 additional human Vκ gene segments, from 5' to 3', included a 5' homology arm containing ~20 kb of mouse genomic sequence 5' of the heavy chain locus, a first

recombinase recognition site (R1), a hygromycin cassette, a second recombinase recognition site (R2) and ~140 kb of human genomic κ sequence containing 12 consecutive human Vλ gene segments, ~31 kb of which overlaps with the 5' end of the human κ sequence of Targeting Vector 1 and serves as the 3' homology arm for this targeting construct. Homologous recombination with this targeting vector created a modified mouse heavy chain locus containing 16 human Vκ gene segments and five human Jκ gene segments operably linked to the mouse heavy chain constant genes which, upon recombination, leads to the formation of a hybrid heavy chain.

[00506] Introduction of fourteen additional human Vκ gene segments into a hybrid heavy chain locus. A third targeting vector was engineered for introduction of 14 additional human Vκ gene segments to the modified mouse heavy chain locus described above (FIG. 2, Targeting Vector 3). A 160,579 bp human genomic fragment containing 15 consecutive human Vκ gene segments was engineered with a 5' homology arm containing mouse genomic sequence 5' of the mouse heavy chain locus and a 3' homology arm containing human genomic κ sequence. Upstream (5') from the human Vκ2-30 gene segment was an additional 14,687 bp of human κ sequence before the start of the 5' mouse homology arm, which was the same 5' homology used for the previous two targeting vectors (described above, see also FIG. 2). Between the 5' homology arm and the beginning of the human κ sequence was a neomycin cassette flanked by recombinase recognition sites. The 3' homology arm included a 21,275 bp overlap of human genomic κ sequence corresponding to the equivalent 5' end of the ~140 kb fragment of human genomic κ sequence of Targeting Vector 2 (FIG. 20). The final targeting vector for the insertion of 14 additional human Vκ gene segments, from 5' to 3' included a 5' homology arm containing ~20 kb of mouse genomic sequence 5' of the mouse heavy chain locus, a first recombinase recognition site (R1), a neomycin cassette, a second recombinase recognition site (R2) and ~161 kb of human genomic κ sequence containing 15 human Vκ gene segments, ~21 kb of which overlaps with the 5' end of the human κ sequence of Targeting Vector 2 and serves as the 3' homology arm for this targeting construct. Homologous recombination with this targeting vector created a modified mouse heavy chain locus containing 30 human Vκ gene segments and five human Jκ gene segments operably linked to the mouse heavy chain constant genes which, upon recombination, leads to the formation of a chimeric κ heavy chain.

[00507] Introduction of ten additional human Vκ gene segments into a hybrid heavy chain locus. A fourth targeting vector was engineered for introduction of 10 additional human Vκ gene segments to the modified mouse heavy chain locus described above (FIG. 2, Targeting Vector 4). A 90,398 bp human genomic fragment containing 16 consecutive

human Vκ gene segments was engineered with a 5' homology arm containing mouse genomic sequence 5' of the mouse heavy chain locus and a 3' homology arm containing human genomic κ sequence. Upstream (5') from the human Vκ2-40 gene segment was an additional 8,484 bp of human κ sequence before the start of the 5' mouse homology arm, which was the same 5' homology as the previous targeting vectors (described above, FIG. 2). Between the 5' homology arm and the beginning of the human κ sequence was a hygromycin cassette flanked by recombinase recognition sites. The 3' homology arm included a 61,615 bp overlap of human genomic κ sequence corresponding to the equivalent 5' end of the ~160 kb fragment of human genomic κ sequence of Targeting Vector 3 (FIG. 2). The final targeting vector for the insertion of 10 additional human Vκ gene segments, from 5' to 3', included a 5' homology arm containing ~20 kb of mouse genomic sequence 5' of the mouse heavy chain locus, a first recombinase recognition site (R1), a hygromycin cassette, a second recombinase recognition site (R2) and ~90 kb of human genomic κ sequence containing 16 human Vκ gene segments, ~62 kb of which overlaps with the 5' end of the human κ sequence of Targeting Vector 3 and serves as the 3' homology arm for this targeting construct. Homologous recombination with this targeting vector created a modified mouse heavy chain locus containing 40 human Vκ gene segments and five human Jκ gene segments operably linked to the mouse heavy chain constant genes which, upon recombination, leads to the formation of a chimeric κ heavy chain (bottom of FIG. 2).

[00508] Using a similar approach as described above, other combinations of human light chain variable domains in the context of mouse heavy chain constant regions are constructed. Additional light chain variable domains may be derived from human Vλ and Jλ gene segments (FIG. 3 and FIG. 4).

[00509] The human λ light chain locus extends over 1,000 kb and contains over 80 genes that encode variable (V) or joining (J) segments. Among the 70 Vλ gene segments of the human λ light chain locus, anywhere from 30-38 appear to be functional gene segments according to published reports. The 70 Vλ sequences are arranged in three clusters, all of which contain different members of distinct V gene family groups (clusters A, B and C). Within the human λ light chain locus, over half of all observed Vλ domains are encoded by the gene segments 1-40, 1-44, 2-8, 2-14, and 3-21. There are seven Jλ gene segments, only four of which are regarded as generally functional Jλ gene segments—Jλ1, Jλ2, Jλ3, and Jλ7. In some alleles, a fifth Jλ-Cλ gene segment pair is reportedly a pseudo gene (Cλ6). Incorporation of multiple human Jλ gene segments into a hybrid heavy chain locus,

as described herein, is constructed by de novo synthesis. In this way, a genomic fragment containing multiple human Jλ gene segments in germline configuration is engineered with multiple human Vλ gene segments and allow for normal V-J recombination in the context of a heavy chain constant region.

[00510] Coupling light chain variable domains with heavy chain constant regions represents a potentially rich source of diversity for generating unique V_L binding proteins with human V_L regions in non-human animals. Exploiting this diversity of the human λ light chain locus (or human κ locus as described above) in mice results in the engineering of unique hybrid heavy chains and gives rise to another dimension of binding proteins to the immune repertoire of genetically modified animals and their subsequent use as a next generation platform for the generation of therapeutics.

[00511] Additionally, human D_H and J_H (or Jκ) gene segments can be incorporated with either human Vκ or Vλ gene segments to construct novel hybrid loci that will give rise, upon recombination, to novel engineered variable domains (FIG. 5 and 6). In this latter case, engineering combinations of gene segments that are not normally contained in a single locus would require specific attention to the recombination signal sequences (RSS) that are associated with respective gene segments such that normal recombination can be achieved when they are combined into a single locus. For example, V(D)J recombination is known to be guided by conserved noncoding DNA sequences, known as heptamer and nonamer sequences that are found adjacent to each gene segment at the precise location at which recombination takes place. Between these noncoding DNA sequences are nonconserved spacer regions that either 12 or 23 base pairs (bp) in length. Generally, recombination only occurs at gene segments located on the same chromosome and those gene segments flanked by a 12-bp spacer can be joined to a gene segment flanked by a 23-bp spacer, *i.e.* the 12/23 rule, although joining two of D_H gene segments (each flanked by 12-bp spacers) has been observed in a small proportion of antibodies. To allow for recombination between gene segments that do not normally have compatible spacers (*e.g.,* Vκ and a D_H or D_H and Jλ), unique, compatible spacers are synthesized in adjacent locations with the desired gene segments for construction of unique hybrid heavy chains that allow for successful recombination to form unique heavy chains containing light chain variable regions.

[00512] Thus, using the strategy outlined above for incorporation of human κ light chain gene segments into an endogenous heavy chain locus allows for the use of other combinations of human λ light chain gene segments as well as specific human heavy chain gene segments (*e.g.,* D_H and J_H) and combinations thereof.

Example 2. Identification of Targeted ES Cells and Generation of Genetically Modified Mice Bearing Human Light Chain Gene Segments at an Endogenous Heavy Chain Locus

[00513] The targeted BAC DNA made in the foregoing Examples is used to electroporate mouse ES cells to created modified ES cells for generating chimeric mice that express V_L binding proteins (*i.e.,* human κ light chain gene segments operably linked to mouse heavy chain constant regions). Targeted ES cells containing an insertion of unrearranged human κ light chain gene segments are identified by a quantitative PCR assay, TAQMAN® (Lie, Y.S., and Petropoulos, C.J. (1998) Advances in quantitative PCR technology: 5' nuclease assays. *Curr Opin Biotechnol* 9(1): 43-48). Specific primers sets and probes are designed to detect insertion of human κ sequences and associated selection cassettes, loss of mouse heavy chain sequences and retention of mouse sequences flanking the endogenous heavy chain locus.

[00514] ES cells bearing the human κ light chain gene segments can be transfected with a construct that expresses a recombinase in order to remove any undesired selection cassette introduced by the insertion of the targeting construct containing human κ gene segments. Optionally, mice bearing an engineered heavy chain locus containing the human κ light chain gene segments can be bred to a FLPe deletor mouse strain (see, *e.g.*, Rodriguez, C.I. *et al.* (2000) High-efficiency deletor mice show that FLPe is an alternative to Cre-loxP. *Nature Genetics* 25: 139-140; US 6,774,279) in order to remove any Frt'ed cassette introduced by the targeting vector that is not removed, *e.g.*, at the ES cell stage or in the embryo. Optionally, the selection cassette is retained in the mice.

[00515] 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 modified heavy chain locus bearing human Vκ and Jκ gene segments operably linked to the mouse immunoglobulin heavy chain constant region genes are identified by genotyping using a modification of allele assay (Valenzuela et al., *supra*) that detected the presence and/or absence of cassette sequences, the human Vκ and Jκ gene segments and endogenous heavy chain sequences.

[00516] Pups are genotyped and a pup heterozygous for a modified heavy chain locus containing human κ light chain gene segments operably linked to the endogenous mouse immunoglobulin heavy chain constant genes is selected for characterizing the immunoglobulin heavy chain repertoire.

Example 3. Propagation of Mice Expressing V_L Binding Proteins

[00517] To create a new generation of V_L binding proteins, mice bearing the unrearranged human κ gene segments can be bred to another mouse containing a deletion of the opposite or untargeted endogenous heavy chain allele (*i.e.,* a mouse heterozygous for the modification). In this manner, the progeny obtained would express only hybrid heavy chains as described in Example 1. Breeding is performed by standard techniques recognized in the art and, alternatively, by commercial companies, *e.g.*, The Jackson Laboratory. Mouse strains bearing a modified heavy chain locus are screened for presence of the unique heavy chains containing human light chain variable domains.

[00518] Alternatively, mice bearing the unrearranged human κ gene segments at the mouse heavy chain locus can be optimized by breeding to other mice containing one or more deletions in the mouse light chain loci (κ and λ). In this manner, the progeny obtained would express unique human κ heavy chain only antibodies as described in Example 1. Breeding is similarly performed by standard techniques recognized in the art and, alternatively, by commercial companies, *e.g.*, The Jackson Laboratory. Mouse strains bearing a modified heavy chain locus and one or more deletions of the mouse light chain loci are screened for presence of the unique heavy chains containing human Vκ domains and mouse heavy chain constant domains and absence of endogenous light chains.

[00519] Mice bearing a modified heavy chain locus (described above) are also bred with mice that contain a replacement of the endogenous κ light chain variable gene locus with the human κ light chain variable gene locus (see US 6,596,541, Regeneron Pharmaceuticals, The VELOCIMMUNE® Humanized Mouse Technology). The VELOCIMMUNE® Humanized Mouse includes, in part, having a genome comprising human κ light chain variable regions operably linked to endogenous κ light chain variable constant region loci such that the mouse produces antibodies comprising a human κ light chain variable domain and a mouse heavy chain constant domain in response to antigenic stimulation. The DNA encoding the variable regions of the light chains of the antibodies can be isolated and operably linked to DNA encoding the human light chain constant regions. The DNA can then be expressed in a cell capable of expressing the fully human light chain of the antibody. Upon a suitable breeding schedule, mice bearing a replacement of the endogenous κ light chain with the human κ light chain locus and a modified heavy chain locus according to Example 1 are obtained. Unique V_L binding proteins containing somatically mutated human Vκ domains can be isolated upon immunization with an antigen of interest.

Example 4. Reengineering of ADAM Genes into a Modified Heavy Chain Locus

[00520] Mice with modified immunoglobulin heavy chain loci in which the endogenous variable region gene segments (*i.e.,* VDJ) have been replaced and/or deleted lack expression of endogenous ADAM6 genes. In particular, male mice comprising such modifications of the immunoglobulin heavy chain loci demonstrate a reduction in fertility. This Example demonstrates two methods to reengineer the capability to express ADAM6 into the mice with the modified heavy chain loci according to Example 1, thus allowing for the maintenance of the modified mouse strains using normal breeding methods.

[00521] Reengineering of ADAM6 Genes Within Human Light Chain Gene Segments. A modified immunoglobulin heavy chain locus containing human Vκ and Jκ gene segments was reengineered to contain a genomic fragment encoding mouse ADAM6a and ADAM6b 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 human Vκ and Jκ gene segments contiguous with mouse ADAM6 genes and mouse heavy chain constant regions.

[00522] A mouse BAC clone (VI149) containing, from 5' to 3', a unique restriction site (I-CeuI), mouse Adam6a and Adam6b genes, an IGCR1 regulatory element (Guo *et al.,* 2011), immunoglobulin D_H and J_H gene segments, an Eμ enhancer, and an IgM constant region gene was used as starting material for reengineering ADAM6 genes in to a modified heavy chain locus containing V_L and J_L gene segments (FIG. 7). VI149 was modified by bacterial homologous recombination (BHR) to delete all of the D_H and J_H gene segments and the IgM gene from about 53 bp 5' of the most distal D segment (DFL16.1) to the 3' end of the BAC. This region was replaced by a spectinomycin-resistance cassette (pSVi0029) that contained a unique AscI site at its 5' end to yield the BAC clone VI413.

[00523] Additional BHR modifications were made to create BAC clones containing the mouse Adam6a and Adam6b genes, as well as the IGCR1 element. The first BAC clone was created by replacing a 47199 bp region between Adam6a and Adam6b with a Frt'ed neomycin-resistance cassette with unique I-CeuI (5') and AscI (3') restriction sites (pLMa0294). This deletion spanned the region from 4779 bp 3' of the Adam6b CDS to 290 bp 5' of the Adam6b CDS. The resulting BAC clone was named VI421. The second BAC clone was created by inserting the same Frt'ed cassette between Adam6a and Adam6b at a position 4782 bp 3' of the Adam6a CDS in VI413 to yield VI422.

[00524] The VI421 BAC clone contained, from 5' to 3', a unique I-CeuI site, Adam6a including 751 bp 5' and 4779 bp 3' of the CDS, the Frt'ed neomycin-resistance cassette,

Adam6b including 290 bp 5' and 7320 bp 3' of the CDS, IGCR1, and a unique AscI site (SEQ ID NO: 3).

[00525] The VI422 BAC clone contained, from 5' to 3', a unique I-CeuI site, Adam6a including 751 bp 5' and 4779 bp 3' of the CDS, the Frt'ed hygromycin-resistance cassette, Adam6b including 47490 bp 5' and 7320 bp 3' of the CDS, IGCR1, and a unique AscI site (SEQ ID NO: 4).

[00526] Reengineering of ADAM6 genes was accomplished by insertion of VI421 and VI422 into the intergenic region of modified version of Targeting Vector 1 (FIG. 2) as described in Example 1. Targeting Vector 1 was modified by two BHR steps to insert the mouse ADAM6 fragments from VI421 and VI422. The first BHR step, the neomycin cassette from Targeting Vector 1 was deleted with a hygromycin cassette (pLMa0100). The resulting BAC clone was named VI425, which contained, from 5' to 3', a hygromycin-resistance cassette, the four most proximal human Vκ segments, a 23,552 bp Vκ-Jκ intergenic region, and all five human Jκ segments, which are functionally linked to an 8 kb 3' mouse homology arm containing the mouse Eμ enhancer and IgM constant region gene. For the second BHR, VI425 was modified to replace 740 bp within the Vκ-Jκ intergenic region with a chloramphenicol-resistance cassette flanked by unique I-CeuI and AscI restriction sites (pDBa0049; FIG. 8). The location of the 740 bp deletion was from 16,858 to17,597 bp 3' of the most proximal human Vκ gene segment (Vκ4-1). The resulting BAC clone from both BHRs was named VI426 (FIG. 8).

[00527] The DNA fragment containing mouse ADAM6 genes from VI421 and VI422 were independently used to replace the chloramphenicol cassette of VI426 by I-CeuI/AscI digestion and relegation of compatible ends. FIG. 8 shows the final targeting vectors, named VI429 and VI428, respectively. Each were used to electroporate into ES cells previously modified with Targeting Vector 4 (as described in Example 1, see FIG. 2) to allow for recombination with the unique heavy chain locus modified in accordance with Example 1 and insertion of the DNA fragment encoding mouse ADAM6 genes. Positive colonies were selected with neomycin.

[00528] Reengineering of ADAM6 Genes Flanking Human Light Chain Gene Segments. A modified immunoglobulin heavy chain locus containing human Vκ and Jκ gene segments located upstream of all the endogenous heavy chain constant regions was reengineered to contain a genomic fragment encoding mouse ADAM6a and ADAM6b 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

human Vκ and Jκ gene segments contiguous with mouse ADAM6 genes and mouse heavy chain constant regions.

[00529] Targeting Vector 4 made in accordance with Example 1 (see FIG. 2 and top of FIG. 9) was modified by BHR to replace the Frt'ed hygromycin cassette with a chloramphenicol cassette containing unique AscI (5') and I-CeuI (3') restriction sites (pLMa0231; FIG. 9). Targeting Vector 4 contained, from 5' to 3', an ~20 kb mouse distal IgH homology arm, a Frt'ed hygromycin-resistance cassette, and human Vκ2-40 to human Vκ3-25 gene segments.

[00530] Next, a BAC clone named VI444 was used to insert a DNA fragment encoding mouse ADAM6 genes at a position 5' of the human Vκ gene segments of the VI477 BAC clone by AscI/I-CeuI digestion and relegation of compatible ends. The VI444 clone contained, from 5' to 3', a unique I-CeuI site, the Adam6a gene including 751 bp 5' and 4779 bp 3' of the CDS, a Frt'ed neomycin-resistance cassette, the Adam6b gene including 290 bp 5' and 1633 bp 3' of the CDS, and a unique AscI site (SEQ ID NO: 5). The resulting BAC clone used as the targeting vector for insertion of mouse ADAM6 genes upstream of human Vκ gene segments was named VI478, which, in contrast to VI421 and VI422, positioned the mouse ADAM6 genes in VI478 are in reverse orientation (*i.e.,* the same transcriptional direction relative to the human Vκ gene segments; FIG. 9). The final targeting vector for insertion of mouse ADAM6 genes at the distal end of the human Vκ gene segments contained, from 5' to 3', the ~20 kb mouse distal IgH homology arm, a unique AscI site, mouse Adam6b, a Frt'ed neomycin-resistance cassette, mouse Adam6a, a unique I-CeuI site, and human Vκ2-40 to human Vκ3-25 gene segments. This targeting vector was used to electroporate into ES cells previously modified with Targeting Vector 4 (FIG. 2) to allow for recombination with the unique heavy chain locus modified in accordance with Example 1 and insertion of the DNA fragment encoding mouse ADAM6 genes. Positive colonies were selected with neomycin.

[00531] **Selection and Confirmation of targeted ES cells.** Each of the final targeting vectors (described above) were used to electroporate mouse ES cells to create modified ES cells comprising a mouse genomic sequence ectopically placed that comprises mouse ADAM6a and ADAM6b sequences within modified heavy chain locus containing human Vκ and Jκ gene segments. Positive ES cells containing the ectopic mouse genomic fragment within the modified heavy chain locus were identified by a quantitative PCR assay using TAQMAN™ probes (Lie and Petropoulos (1998), *supra*).

[00532] 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; and 7,294,754). Mice bearing a modified heavy chain locus containing human κ light chain 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 modified heavy chain locus as well as human κ light chain sequences.

[00533] Pups are genotyped and a pup heterozygous for a modified 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 non-human animal comprising

(a) an insertion of one or more human V_L and one or more human J_L gene segments upstream of an non-human immunoglobulin light chain constant region,

(b) an insertion of one or more human V_L and one or more human J_L gene segments upstream of an non-human immunoglobulin heavy chain constant region, and

(c) a nucleotide sequence that encodes an ADAM6 protein or a functional fragment thereof, wherein the ADAM6 protein is expressed from an ectopic ADAM6 nucleic acid sequence.

2. The non-human animal of claim 1, wherein the non-human heavy and/or light chain constant regions are rodent constant regions.

3. The non-human animal of claim 1 or 2, wherein the light chain constant region is a mouse Cκ.

4. The non-human animal of any one of the preceding claims, wherein the human V_L and J_L gene segments upstream of the non-human immunoglobulin light chain constant region are human Vκ and Jκ gene segments.

5. The non-human animal of any one of the preceding claims, wherein the human V_L and J_L gene segments upstream of the non-human immunoglobulin heavy chain constant region are human Vκ and Jκ gene segments.

6. The non-human animal of any one of the preceding claims, wherein the nucleotide sequence that encodes an ADAM6 protein or functional fragment thereof is present the same location as compared to a wild-type type non-human ADAM6 locus.

7. The non-human animal of any one of the preceding claims, wherein the nucleotide sequence that encodes an ADAM6 protein or functional fragment thereof is present within immunoglobulin gene segments.

8. The non-human animal of claim 7, wherein the immunoglobulin gene segments are human κ light chain gene segments.

9. The non-human animal of claim 7 or 8, wherein the immunoglobulin gene segments are endogenous heavy chain gene segments of the non-human animal.

10. The non-human animal of any one of the preceding claims, wherein the non-human animal comprises endogenous immunoglobulin V_L and/or J_L gene segments that are incapable of rearranging to form an immunoglobulin V_L domain in the non-human animal.

11. A genetically modified non-human animal that comprises

(a) one or more unrearranged human Vκ gene segments and one or more unrearranged human Jκ gene segments at an endogenous immunoglobulin heavy chain locus of the non-human animal,

(b) one or more unrearranged human Vκ gene segments and one or more unrearranged human Jκ gene segments at an endogenous immunoglobulin light chain locus of the non-human animal, wherein the non-human animal is capable of expressing an ADAM6 protein or functional fragment thereof.

12. A cell derived from the non-human animal of claim 1 or 11.

13. The cell of claim 12, wherein the cell is a B cell.

14. A hybridoma made from the B cell of claim 13.

15. The cell of claim 12, wherein the non-human animal is a rodent.

16. The cell of claim 15, wherein the rodent is selected from a mouse and a rat.

17. A method for making an antigen-binding protein that binds to an antigen of interest is provided, wherein the method comprises

(a) exposing a non-human animal of claim 1 or 11 to an antigen of interest,

(b) isolating one or more B lymphocytes of the non-human animal, wherein the one or more B lymphocytes express a V_L binding protein that binds the antigen of interest, and

(c) identifying a nucleic acid sequence that encodes a V_L domain of the V_L binding protein that binds that antigen of interest, wherein the V_L binding protein comprises a human Vκ domain and a non-human light chain constant domain and a human Vκ domain and a non-human heavy chain constant domain, and

(d) employing the nucleic acid sequence of (c) with a human immunoglobulin constant region nucleic acid sequence to make a human antigen-binding protein that binds the antigen of interest.

18. The method of claim 17, wherein the non-human light chain constant region of the V_L binding protein is a mouse Cκ and the non-human heavy chain constant region is a mouse heavy chain constant region.

19. The method of claim 17 or 18, wherein the non-human animal is a mouse.

20. A rodent comprising one or more human immunoglobulin light chain gene segments operably linked to a non-human immunoglobulin heavy chain constant region gene, wherein the rodent expresses one or more ADAM6 proteins.

21. The rodent of claim 20, wherein the one or more human immunoglobulin light chain gene segments are human Vκ and Jκ gene segments.

22. The rodent of claim 20 or 21, wherein the non-human immunoglobulin heavy chain constant region gene is a mouse or rat heavy chain constant region gene.

23. The rodent of any one of claims 20 – 22, wherein the immunoglobulin heavy chain constant region gene comprises a C_H1 and/or a hinge region.

24. The rodent of any one of claims 20 – 23, wherein the rodent comprises deletion of, or replacement of, one or more endogenous immunoglobulin heavy chain gene sequences.

25. The rodent of any one of claims 20 – 24, wherein the endogenous V_H, D_H, and J_H gene segments are incapable of rearranging to form a rearranged V/D/J sequence.

26. The rodent of any one of claims 20 – 25, wherein the rodent comprises a deletion of an endogenous ADAM6 gene and further comprises an ectopic mouse ADAM6 gene.

27. The rodent of any one of claims 20 – 26, wherein the rodent is selected from a mouse or a rat.

28. A method for modifying a heavy chain immunoglobulin locus of a rodent, comprising:

(a) making a first modification of a rodent heavy chain immunoglobulin locus that results in a reduction or elimination of endogenous ADAM6 activity in a male rodent, wherein the first modification includes insertion a gene segment selected from one or more human V_L gene segments, one or more human J_L gene segments, and a combination thereof; and,

(b) making a second modification to add a nucleic acid sequence to the rodent that confers upon the rodent ADAM6 activity that is functional in a male rodent.

29. The method according to claim 28, wherein the first modification further comprises the insertion of one or more human D_H gene segments that are capable of rearranging with one or more V_L and one or more J_L gene segments.

30. The method according to claim 28, wherein the one or more V_L gene segments and one or more human J_L gene segments are $V\kappa$ and $J\kappa$ gene segments or $V\lambda$ and $J\lambda$ gene segments.

31. The method according to any one of claims 28 – 30, wherein the nucleic acid sequence that confers upon the rodent ADAM6 activity that is functional in a male rodent is contiguous with the one or more human V_L gene segments and/or the one or more human J_L gene segments.

32. The method according to any one of claims 28 – 31, wherein the first and the second modification are made in a single ES cell, and the single ES cell is introduced into a host embryo to make the rodent.