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DESCRIPTION

Description

RELATED APPLICATION

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application serial number 62/345,524, filed June 3, 2016.

BACKGROUND

[0002] Non-human animals, particularly mice and rats, have proven to be a valuable source of therapeutic antibodies and potentially could serve as a source of other antigen binding molecules. A high level of antigen receptor diversity in such non-human animals increases the likelihood that an antigen binding molecule having desirable therapeutic properties will be generated following immunization. Accordingly, there is a need for genetically engineered non-human animals that have increased antigen receptor diversity to improve production of therapeutic antigen binding molecules.

SUMMARY

[0003] The invention provides a method of making an antibody specific for an antigen, wherein the method comprises exposing a genetically modified rodent to the antigen, wherein the genetically modified rodent comprises in its genome: a nucleic acid sequence encoding a human Terminal Deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element that drives expression of the nucleic acid sequence encoding the human TdT in pre-B cells; and a human immunoglobulin variable region specific for the antigen comprising unrearranged human immunoglobulin variable region gene segments, wherein the human immunoglobulin variable region is operably linked to an immunoglobulin constant region.

The invention also provides a method of making a nucleic acid encoding a human immunoglobulin heavy chain variable domain specific for an antigen and/or a human immunoglobulin light chain variable domain specific for an antigen, wherein the method comprises: (a) exposing a genetically modified rodent to the antigen, wherein the genetically modified rodent comprises in its genome: a nucleic acid sequence encoding a human Terminal Deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element that drives expression of the nucleic acid sequence encoding the human TdT in pre-B cells; and a human immunoglobulin variable region specific for the antigen comprising unrearranged human immunoglobulin variable region gene segments, wherein the human immunoglobulin variable region is operably linked to an immunoglobulin constant region; and (b) obtaining a nucleic acid sequence encoding a human immunoglobulin heavy chain variable domain specific for the antigen and/or a human immunoglobulin light chain variable domain specific for the antigen from the rodent. In certain aspects, described herein are genetically modified rodents comprising in their genome an exogenous human nucleic acid encoding terminal deoxynucleotidyltransferase (TdT).TdT.. In some aspects, the rodents described herein express the human TdT encoded by the exogenous nucleic acid during B cell development, for example, in pro-B cells and/or in pre- B cells. In some aspects, the genetically modified non-human animal comprises multiple copies of exogenous nucleic acids encoding TdT (*e.g.,* at least 2, 3, 4, 5, 6, 7 or 8 copies).

[0004] In some aspects, the genetically modified rodent comprises in its genome a human immunoglobulin variable region specific for the antigen comprising unrearranged human immunoglobulin variable region gene segments (*e.g.*, heavy chain gene segments, κ chain gene segments, λ chain gene segments) operably linked to an immunoglobulin constant region gene (*e.g.,* a heavy chain constant region gene, a κ chain constant region gene, a λ chain constant region gene). In some aspects, the constant region gene is a human constant region gene, a mouse constant region gene or a rat constant region gene. In some aspects, the constant region gene is of endogenous species origin. In some aspects, the variable region and the constant region gene are located in an endogenous immunoglobulin locus (*e.g.,* a heavy chain locus, a κ locus, a λ locus). In some aspects, the genetically modified rodent expresses antibodies comprising a human immunoglobulin variable domain derived from the immunoglobulin variable region and an immunoglobulin constant domain encoded by the immunoglobulin constant region gene. In some aspects, provided herein are methods of using such a genetically modified rodent to generate an antibody, a B cell, a hybridoma or a nucleic acid encoding a human immunoglobulin variable domain.

[0005] In certain aspects, the genetically modified rodent comprises in its genome a T cell receptor (TCR) variable region comprising unrearranged human TCR variable region gene segments (*e.g.*, TCRα gene segments, TCR β gene segments, TCRγ gene segments, TCRδ gene segments) operably linked to a TCR constant region gene (*e.g.,* TCRα constant region gene, TCR β constant region gene, TCRγ constant region gene, TCRδ constant region gene). In some aspects, the constant region gene is a human constant region gene, a mouse constant region gene or a rat constant region gene. In some aspects, the constant region gene is of endogenous species origin. In some aspects, the variable region and the constant region gene are located in an endogenous TCR locus (*e.g.,* TCRα locus, TCR β locus, TCRγ locus, TCRδ locus). In some aspects, the genetically modified rodent expresses TCR comprising a human TCR variable domain derived from the TCR variable region and a TCR constant domain encoded by the TCR constant region gene. In some aspects, provided herein are methods of using such a genetically modified rodent to generate a TCR, a T cell, a T cell hybridoma or a nucleic acid encoding a human TCR variable domain.

[0006] In some aspects, the genetically modified rodent comprises in its genome an immunoglobulin variable region comprising unrearranged human immunoglobulin variable region gene segments (*e.g.*, heavy chain gene segments, κ chain gene segments, λ chain gene segments) operably linked to a TCR constant region gene (*e.g.,* TCRα constant region gene, TCR β constant region gene, TCRγ constant region gene, TCRδ constant region gene). In some aspects, the constant region gene is a human constant region gene, a mouse constant region gene or a rat constant region gene. In some aspects, the constant region gene is of endogenous species origin. In some aspects, the variable region and the constant region gene are located in an endogenous TCR locus (*e.g.,* TCRα locus, TCR β locus, TCRγ locus, TCRδ locus). In some aspects, the genetically modified rodent expresses chimeric antigen receptor (CAR) comprising a human immunoglobulin variable domain derived from the immunoglobulin variable region and a TCR constant domain encoded by the TCR constant region gene. In some aspects, provided herein are methods of using such a genetically modified rodent to generate an CAR, a T cell, a T cell hybridoma or a nucleic acid encoding a human immunoglobulin variable domain.

[0007] In some aspects, provided herein are methods of making a rodent disclosed herein comprising engineering the non-human animal to comprise in its germline the genetic modifications described herein. In some aspects, provided herein are non-human ES cells comprising the genetic modifications described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008]

Figure 1 depicts a diagram of an exemplary targeting vector (not to scale) whereas part of the mouse Rag2 gene is replaced with a DNA sequence encoding short isoform human TdT (hTdTs). In exemplary aspects, the vector is randomly integrated into the genome. Unless labeling in the diagram suggests otherwise (*e.g.*, as for selection cassettes, loxP sites, *etc.*), filled shapes and single lines represent mouse sequences, and empty shapes and double lines represent human sequences. E1, E2, etc. represent exons of particular illustrated genes, GFP is green fluorescent protein, CM is chloramphenicol resistant gene, neo is neomycin resistant gene. Junctions 1-4 correspond to junctions indicated in Table 1.

Figure 2 depicts a diagram of an exemplary targeting vector (not to scale) where a part of the mouse Rag2 gene is replaced with a DNA sequence encoding short isoform human TdT (hTdTs). In the depicted aspect, the vector is used to insert hTdT, driven by the mouse RAG2 promoter, into the Ig kappa locus. Unless labeling in the diagram suggests otherwise (*e.g.,* as for selection cassettes, loxP sites, *etc.*), filled shapes and single lines represent mouse sequences, and empty shapes and double lines represent human sequences. E1, E2, etc. represent exons of particular illustrated genes, GFP is green fluorescent protein, CM is chloramphenicol resistant gene, hyg is hygromycin resistant gene. Junctions 1-7 correspond to junctions in Table 2.

Figure 3 depicts a diagram of an exemplary targeting vector (not to scale) used to insert a

DNA sequence encoding human TdT (hTdTs), driven by V_H1-72 promoter and Eμ enhancer, into the immunoglobulin κ locus. Unless labeling in the diagram suggests otherwise (*e.g.,* as for selection cassettes, loxP sites, *etc.*), filled shapes and single lines represent mouse sequences, and empty shapes and double lines represent human sequences. E1, E2, etc. represent exons of particular illustrated genes, GFP is green fluorescent protein, CM is chloramphenicol resistant gene, hyg is hygromycin resistant gene. Junctions 1-4 correspond to junctions in Table 3.

Figure 4 depicts expression of hTdT mRNA in lymphocytes of VELOCIMMUNE® TdT mice compared to VELOCIMMUNE® control mice. VELOCIMMUNE® mice herein are mice that comprise a diverse repertoire of unrearranged human heavy chain and kappa light chain variable (V(D)J) gene segments. Het indicates a heterozygous mouse, HO indicates a homozygous mouse.

Figure 5 depicts a graph showing hIgκ sequence diversity (# of unique light chain CDR3 sequences per 10,000 hIgκ sequencing reads) in VELOCIMMUNE® mice expressing hTdT compared to VELOCIMMUNE® control mice. Het indicates a heterozygous mouse, HO indicates a homozygous mouse.

Figure 6 depicts a graph showing the distribution of hIgκ non-template additions in VELOCIMMUNE® mice expressing hTdT compared to VELOCIMMUNE® control mice. Het indicates a heterozygous mouse, HO indicates a homozygous mouse. "NT" stands for nucleotides.

Figure 7 has two panels. Panel (A) depicts a graph showing the distribution of hIgκ CDR3 lengths in VELOCIMMUNE® mice expressing hTdT compared to VELOCIMMUNE® control mice. "AA" stands for amino acid. Panel (B) depicts a graph showing exonuclease deletion length frequencies at 5' region of JK segments in VELOCIMMUNE® mice expressing hTdT compared to VELOCIMMUNE® control mice. Het indicates a heterozygous mouse, HO indicates a homozygous mouse.

Figure 8 has two panels. Panel (A) depicts a graph showing Vκ usage in VELOCIMMUNE® mice expressing hTdT compared to VELOCIMMUNE® control mice. Panel (B) depicts a graph showing Jκ usage in VELOCIMMUNE® mice expressing hTdT compared to VELOCIMMUNE® control mice. Het indicates a heterozygous mouse, HO indicates a homozygous mouse.

Figure 9 depicts a graph showing mIgλ sequence diversity (# of unique light chain CDR3 sequences per 10,000 Igλ sequencing reads) in VELOCIMMUNE® mice expressing hTdT compared to VELOCIMMUNE® control mice. Het indicates a heterozygous mouse, HO indicates a homozygous mouse.

Figure 10 depicts a graph showing the distribution of mIgλ non-template additions in VELOCIMMUNE® mice expressing hTdT compared to VELOCIMMUNE® control mice. Het indicates a heterozygous mouse, HO indicates a homozygous mouse. "NT" stands for nucleotides.

Figure 11 depicts a graph showing the distribution of mIgλ CDR3 lengths in VELOCIMMUNE® mice expressing hTdT compared to VELOCIMMUNE® control mice. Het indicates a heterozygous mouse, HO indicates a homozygous mouse. "AA" stands for amino acid.

Figure 12 depicts a graph showing Vλ usage in VELOCIMMUNE® mice expressing hTdT compared to VELOCIMMUNE® control mice. Het indicates a heterozygous mouse, HO indicates a homozygous mouse.

Figure 13 depicts a graph showing hIgκ sequence diversity (# of unique light chain CDR3 sequences per 10,000 Igκ sequencing reads) in dual light chain mice (DLC; mice comprising two unrearranged human Vk gene segments and five unrearranged human Jk gene segments, as well as a diverse repertoire of unrearranged human heavy chain V, D, and J gene segments) expressing hTdT (right panel; hTdT genes present as indicated) compared to VELOCIMMUNE® mice expressing hTdT (left panel; hTdT genes present as indicated) and DLC and VELOCIMMUNE® control mice that do not express hTdT. Het indicates a heterozygous mouse for hTdT, HO indicates a homozygous mouse for hTdT.

Figure 14 depicts a graph showing the distribution of hIgκ non-template additions in mice expressing hTdT compared to DLC control mice not expressing hTdT (DLC). Het indicates a heterozygous mouse for hTdT, HO indicates a homozygous mouse for hTdT. "NT" stands for nucleotides.

Figure 15 depicts a graph showing the distribution of hIgκ CDR3 lengths in DLC mice expressing hTdT compared to DLC control mice not expressing hTdT. Het indicates a heterozygous mouse for hTdT, HO indicates a homozygous mouse for hTdT.

Figure 16 depicts graphs showing Vκ usage and Jκ usage in DLC mice expressing hTdT compared to DLC control mice not expressing hTdT. Het indicates a heterozygous mouse for hTdT, HO indicates a homozygous mouse for hTdT. Only two different Rag TdT tg (HO) DLC mice where used, which are depicted separately.

DETAILED DESCRIPTION

General

[0009] Described herein are methods and compositions related to rodents (e.g. a mouse or rat) comprising in their genome an exogenous nucleic acid encoding human TdT . In certain aspects, the genome of the rodent comprises further modifications such that it expresses antigen binding molecules having human variable domains (*e.g.*, antibodies, TCRs and/or CARs).

[0010] TdT is a DNA polymerase that catalyzes template-independent addition of nucleotides (N-additions) during junction formation in V(D)J recombination, which leads to an increase in antigen-receptor diversity in B and T lymphocytes. In some aspects, the non-human animals provided herein express increased levels of TdT during B cell development and/or T cell development compared to corresponding non-human animals (*i.e.,* non-human animals of the same species and strain) that do not include in their genome an exogenous nucleic acid encoding TdT. In some aspects, the non-human animals provided herein may express TdT during stages of B cell development and/or T cell development during which corresponding non-human animals that do not include in their genome an exogenous nucleic acid encoding TdT do not express TdT (*e.g.,* during the pre-B cell stage). In some aspects, the genetically modified non-human animals described herein may have increased antigen-receptor diversity (*e.g.*, antibody diversity, TCR diversity and/or CAR diversity) compared to corresponding non-human animals that do not include in their genome an exogenous nucleic acid encoding TdT.

Definitions

[0011] The articles "*a*" and "*an*" are used herein to refer to one or to more than one (*i.e.,* to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0012] The term "*amino acid*" is intended to embrace all molecules, whether natural or synthetic, which include both an amino functionality and an acid functionality and capable of being included in a polymer of naturally-occurring amino acids. Exemplary amino acids include naturally-occurring amino acids; analogs, derivatives and congeners thereof; amino acid analogs having variant side chains; and all stereoisomers of any of the foregoing.

[0013] As used herein, the term "*antibody*" may refer to both an intact antibody and an antigen binding fragment thereof. Intact antibodies are glycoproteins that include at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain includes a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. Each light chain includes a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The term "antibody" also includes single domain antibodies, heavy chain only antibodies, antibodies with light chain variable gene segments on heavy chain, etc.

[0014] The terms "*antigen binding fragment*" and "*antigen-binding portion*" of an antigen binding molecule (*e.g.,* an antibody, a T cell receptor (TCR), a chimeric antigen receptor (CAR)), as used herein, refers to one or more fragments of the antigen binding molecule that retain the ability to bind to an antigen. An antigen binding fragment can include any antibody, TCR or CAR fragment that retains at least a portion of the variable region of an intact antigen binding molecule and is capable of binding to an antigen. Examples of binding fragments encompassed within the term "antigen binding fragment" include, but are not limited to Fab, Fab', $F(ab')_2$, Fv, scFv, disulfide linked Fv, Fd, single-chain antibodies, soluble TCR, single-chain TCR, soluble CAR, single-chain CAR. isolated CDRH3 (antibody or TCR), and other antigen binding fragments that retain at least a portion of the variable region of an intact antigen binding molecule. These antigen binding fragments can be obtained using conventional recombinant and/or enzymatic techniques and can be screened for antigen binding in the same manner as intact antibodies.

[0015] The term "*corresponding*" in reference to a non-human animal, is used to describe the features of a control non-human animal of the same species and comprising the same genetic modifications as a subject non-human except that the subject non-human animal expresses exogenous TdT whereas the corresponding non-human animal does not.

[0016] As used herein, a *"chimeric antigen receptor"* or *"CAR"* refers to an antigen binding protein in that includes an immunoglobulin antigen binding domain (*e.g.*, an immunoglobulin variable domain) and a T cell receptor (TCR) constant domain or a portion thereof. As used herein, a "constant domain" of a TCR polypeptide includes a membrane-proximal TCR constant domain, and may also include a TCR transmembrane domain and/or a TCR cytoplasmic tail. For example, as described herein, the CAR may be a dimer that includes a first polypeptide comprising an immunoglobulin heavy chain variable domain linked to a TCRβ constant domain and a second polypeptide comprising an immunoglobulin light chain variable domain (*e.g.,* a κ or λ variable domain) linked to a TCRα constant domain. In some aspects, the CAR may be a dimer that includes a first polypeptide comprising an immunoglobulin heavy chain variable domain linked to a TCRα constant domain and a second polypeptide comprising an immunoglobulin light chain variable domain (*e.g.,* a κ or λ variable domain) linked to a TCRβ constant domain.

[0017] The phrase "*derived from*" when used concerning a rearranged variable region gene or a variable domain "derived from" an unrearranged variable region and/or unrearranged variable region gene segments refers to the ability to trace the sequence of the rearranged variable region gene or variable domain back to a set of unrearranged variable region gene segments that were rearranged to form the rearranged variable region gene that expresses the variable domain (accounting for, where applicable, splice differences and somatic mutations). For example, a rearranged variable region gene that has undergone somatic mutation does not change the fact that it is derived from the unrearranged variable region gene segments.

[0018] As used herein, the term "*locus*" refers to a region on a chromosome that contains a set of related genetic elements (*e.g.*, genes, gene segments, regulatory elements). For example, an unrearranged immunoglobulin locus may include immunoglobulin variable region gene segments, one or more immunoglobulin constant region genes and associated regulatory elements (*e.g.,* promoters, enhancers, switch elements, *etc.*) that direct V(D)J recombination and immunoglobulin expression, while an unrearranged TCR locus may include TCR variable region gene segments, a TCR constant region gene and associated regulatory elements (*e.g.,* promoters, enhancers, *etc.*) that direct V(D)J recombination and TCR expression. Similarly, an unrearranged CAR locus may include immunoglobulin variable region gene segments, a TCR constant region gene and associated regulatory elements (*e.g.,* promoters, enhancers, *etc.*) that direct V(D)J recombination and CAR expression. A locus can be endogenous or non-endogenous. The term "*endogenous locus*" refers to a location on a chromosome at which a particular genetic element is naturally found.

[0019] Unrearranged variable region gene segments are "*operably linked*" to a contiguous constant region gene if the unrearranged variable region gene segments are capable of rearranging to form a rearranged variable region gene that is expressed in conjunction with the constant region gene as a polypeptide chain of an antigen binding protein.

[0020] The terms "*polynucleotide*", and "*nucleic acid*" are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three-dimensional structure, and may perform any function. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. A polynucleotide may be further modified, such as by conjugation with a labeling component. In all nucleic acid sequences provided herein, U nucleotides are interchangeable with T nucleotides.

[0021] As used herein, "*specific binding*" and "antigen specificity" refers to the ability of an antigen binding molecule (*e.g.,* an antibody, a TCR, a CAR) to bind to a predetermined target, such as a predetermined antigen. Typically, an antigen binding molecule specifically binds to its predetermined target with an affinity corresponding to a K_D of about 10^{-7} M or less, and binds to the predetermined target with an affinity corresponding to K_D that is at least 10 fold less, at least 100 fold less or at least 1000 fold less than its K_D for a non-specific and unrelated target (*e.g.,* BSA, casein). In some embodiments, an antigen binding molecule specifically binds to its predetermined target with an affinity corresponding to a K_D of about 10^{-8} M or less, 10^{-9} M or less or 10^{-10} M or less.

[0022] As used herein, a "*T cell receptor*" or "*TCR*" refers to an antigen binding protein in that includes both a TCR antigen binding domain (*e.g.,* a TCR variable domain) and at least a portion of a TCR constant domain. As used herein, a "constant domain" of a TCR polypeptide includes a membrane-proximal TCR constant domain, and may also include a TCR transmembrane domain and/or a TCR cytoplasmic tail. As described herein, the TCR may be a soluble TCR and does not include a TCR transmembrane domain or a TCR cytoplasmic tail. For example, in some aspects, the TCR may be a dimer that includes a first polypeptide comprising a TCRβ variable domain linked to a TCRβ constant domain (or a fragment thereof) and a second polypeptide comprising a TCRα linked to a TCRα constant domain (or a fragment thereof).

[0023] The term "*unrearranged*" includes the state of an immunoglobulin, TCR or CAR variable region locus or variable region gene segments wherein V gene segments and J gene segments (for heavy or TCRβ variable regions, D gene segments as well) are maintained separately but are capable of being joined to form a rearranged V(D)J gene (a "variable region gene") that comprises a single V, (D), J of the V(D)J repertoire.

Genetically modified Non-Human Animals and ES cells

[0024] In certain aspects, described herein are non-human animals and ES cells which may comprise in their genome an exogenous nucleic acid encoding TdT (*e.g.*, human, mouse or rat TdT). In certain aspects, the genome of the rodents and ES cells may comprise further modifications including, for example, modifications that result in the expression of antigen binding molecules having human variable domains (*e.g.*, antibodies, TCRs and/or CARs).

[0025] The genetically modified rodents and ES cells described herein can be generated using any appropriate method known in the art. For example, rodent ES cells containing targeted genetic modifications can be generated using VELOCIGENE® technology, which is described in U.S. Patent Nos. 6,586,251, 6,596,541, 7,105,348, and Valenzuela et al. (2003) "High-throughput engineering of the mouse genome coupled with high-resolution expression analysis" Nat. Biotech. 21(6): 652-659, and U.S Pat. Pub. No. US 2014/0310828. Targeted modifications can also be made using a CRISPR/Cas system, as described, for example, in U.S. Pat. No. 9,228,208, and U.S. Pub. Nos. US 2015-0159174 A1, US 2016-0060657 A1, US 2015-0376650 A1, US 2015-0376651 A1, US 2016-0046960 A1, US 2015-0376628 A1, and US 2016-0115486 A1. Targeted modifications can also be made using a meganuclease, as described, for example, in U.S. Pat. No. 8,703,485, 8,530,214 and 8,624,000. Non-targeted genetic modifications can be made using standard methods in the art, including, for example, as described in U.S. Pat. No. 6,150,584, 6,114,598, 5,633,425, 7,501,552, 6,235,883, 6,998,514 and 5,776,773.

[0026] ES cells described herein can then be used to generate a rodent using methods known in the art. For example, the mouse ES cells described herein can be used to generate genetically modified mice using the VELOCIMOUSE® method, as described in U.S. Pat. No. 7,294,754 and Poueymirou et al., Nature Biotech 25:91-99 (2007). Rat ES cells can be used to generate modified rats using the method described, e.g., U.S. Pat. Pub. No. US 2014/0310828. Resulting mice or rats can be bread to homozygosity. Multiple distinct modifications can be combined in a single genetically modified organism either by breeding of separately modified animals or by introducing additional modifications into an already modified ES cell (*e.g.,* using the methods described herein).

[0027] For rodents where suitable genetically modifiable ES cells are not readily available, other methods can be employed to make a rodent comprising the genetic modifications described herein. Such methods include, for example, modifying a non-ES cell genome (*e.g.,* a fibroblast or an induced pluripotent cell) and employing nuclear transfer to transfer the modified genome to a suitable cell, such as an oocyte, and gestating the modified cell (*e.g.,* the modified oocyte) in a rodent under suitable conditions to form an embryo.

[0028] In certain aspects, the rodent is a mouse, a rat or a hamster. In some aspects, the rodent is selected from the superfamily Muroidea. In some aspects, the rodent is from a family selected from Calomyscidae (*e.g.,* mouse-like hamsters), Cricetidae (*e.g.,* hamster, New World rats and mice, voles), Muridae (*e.g.*, true mice and rats, gerbils, spiny mice, crested rats), Nesomyidae (*e.g.*, climbing mice, rock mice, white-tailed rats, Malagasy rats and mice), Platacanthomyidae (*e.g.,* spiny dormice), and Spalacidae (*e.g.,* mole rates, bamboo rats, and zokors). In some aspects, the rodent is selected from a true mouse or rat (family Muridae), a gerbil, a spiny mouse, and a crested rat. In some aspects, the mouse is from a member of the family Muridae. In some aspects, the rodent is selected from a mouse and a rat. In some aspects, the rodent is a mouse.

[0029] In some aspects, the rodent is a mouse of a C57BL strain. In some aspects, the C57BL strain is selected from C57BL/A, C57BL/An, C57BL/GrFa, C57BL/KaLwN, C57BL/6, C57BL/6J, C57BL/6ByJ, C57BL/6NJ, C57BL/10, C57BL/10ScSn, C57BL/10Cr, and C57BL/Ola. In some aspects, the rodent is a mouse of a 129 strain. In some aspects, the 129 strain is selected from the group consisting of a strain that is 129P1, 129P2, 129P3, 129X1, 129S1 (*e.g.*, 129S1/SV, 129S1/SvIm), 129S2, 129S4, 129S5, 129S9/SvEvH, 129S6 (129/SvEvTac), 129S7, 129S8, 129T1, 129T2. In some aspects, the genetically modified mouse is a mix of a 129 strain and a C57BL strain. In some aspects, the mouse is a mix of 129 strains and/or a mix of C57BL/6 strains. In some aspects, the 129 strain of the mix is a 129S6 (129/SvEvTac) strain. In some aspects, the mouse is a BALB strain (*e.g.*, BALB/c). In some aspects, the mouse is a mix of a BALB strain and another strain (*e.g.,* a C57BL strain and/or a 129 strain). In some aspects, the rodents described herein can be a mouse derived from any combination of the aforementioned strains.

[0030] In some aspects, the rodent described herein is a rat. In some aspects, the rat is selected from a Wistar rat, an LEA strain, a Sprague Dawley strain, a Fischer strain, F344, F6, and Dark Agouti. In some aspects, the rat strain is a mix of two or more strains selected from the group consisting of Wistar, LEA, Sprague Dawley, Fischer, F344, F6, and Dark Agouti.

Rodents expressing exogenous TdT

[0031] In certain aspects, described herein are genetically modified rodentsand ES cells comprising in their germline and/or genome a nucleic acid sequence encoding an exogenous Terminal Deoxynucleotidyltransferase (TdT). Deoxynucleotidyltransferase (TdT) is a DNA polymerase that catalyzes template-independent addition of nucleotides (NP-additions) during junction formation in V(D)J recombination, which leads to an increase in antigen-receptor diversity in B and T lymphocytes. Template-independent additions, non-template additions, and non-germline additions all refer to nucleotide additions catalyzed by TdT, and these terms are used herein interchangeably.

[0032] The sequence of the exogenous TdT in the genome of rodent is human. In some aspects, the nucleic acid sequence is the genomic TdT sequence (*i.e.,* including exons and introns). In some aspects, the nucleic acid sequence encodes TdT mRNA/cDNA (*i.e.,* the exons of one or more TdT isoforms).

[0033] Human TdT (hTdT) is encoded by the DNTT gene, which is located on human chromosome 10. An exemplary genomic DNA sequences of hTdT can be found at position 96304328 to 96338564 of NCBI accession number NC_000010.11. Exemplary mRNA sequence of isoforms of hTdT is provided by NCBI accession numbers NM_001017520.1 and NM_004088.3. The protein sequences encoded by these isoforms is provided by NCBI accession numbers NP_001017520.1 and NP_004079.3, respectively. Among the TdT isoforms is a short isoform (hTdTS) and two long isoforms (hTdTL1 and hTdTL2). The sequences of the three isoforms are provided, for example, in Thai and Kearney, Adv. Immunol. 86:113-36 (2005). In certain aspects the exogenous nucleic acid sequence encodes hTdTS. In some aspects, the exogenous nucleic acid sequence encodes hTdTL1. In some aspects, the exogenous nucleic acid sequence encodes hTdTL2. In certain aspects, the rodent comprises exogenous nucleic acid sequences encoding multiple isoforms (*e.g.*, both hTdTS and hTdTL2). In certain aspects, the rodent comprises exogenous nucleic acid sequences encoding all three human isoforms (*e.g.*, both hTdTS and hTdTL2).

[0034] Mouse TdT (mTdT) is encoded by the Dntt gene, which is located on mouse chromosome 19. An exemplary genomic DNA sequence of mTdT can be found at position 41029275 to 41059525 of NCBI accession number NC_000085.6. Exemplary mRNA sequences of isoforms of mTdT is provided by NCBI accession numbers NM_001043228.1 and NM_009345.2. The protein sequences encoded by these isoforms is provided by NCBI accession numbers NP_001036693.1and NP_033371.2, respectively.

[0035] Rat TdT (rTdT) is encoded by the Dntt gene, which is located on rat chromosome 1. An exemplary genomic DNA sequence of rTdT can be found at position 260289626 to 260321174 of NCBI accession number NC_005100.4. An exemplary mRNA sequence of rTdT is provided by NCBI accession number NM_001012461.1. The protein sequence encoded by this mRNA is provided by NCBI accession number NP_001012479.1.

[0036] In some aspects, the genome of the genetically modified non-human animal contains multiple copies of the nucleic acid sequence encoding the exogenous TdT. In some aspects, the genetically modified non-human animal contains 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 copies of the nucleic acid sequence encoding the exogenous TdT. In some aspects, the genetically modified non-human animal contains at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 copies of the nucleic acid sequence encoding the exogenous TdT.

[0037] In some aspects, the nucleic acid sequence encoding the human TdT is operably linked to one or more transcriptional control element (*e.g.,* a promoter and/or an enhancer). In some aspects, the transcriptional control element is a constitutive (*i.e.,* ubiquitous) promoter. Examples of constitutive promoters include, but are not limited to, a SV40, a CMV promoter, an adenoviral promoter, an EF1 promoter, a β-actin promoter, an EGR1 promoter, an eIF4A1 promoter, a FerH promoter, a FerL promoter, a GAPDH promoter, a GRP78 promoter, a GRP94 promoter, a HSP70 promoter, a β-Kin promoter, a PGK-1 promoter, a ROSA promoter and an Ubiquitin B promoter. In some aspects, the nucleic acid sequence is not operably linked to a constitutive promoter.

[0038] In some aspects, the transcriptional control element induces expression of the encoded TdT during B cell development. In some aspects, the transcriptional control element induces expression of TdT in pro-B cells and/or pre-B cells. In some aspects, the transcriptional control element is a transcriptional control element (*e.g.,* a promoter and/or enhancer) of a gene expressed during B cell development, in pro-B cells and/or in pre-B cells. In some aspects, the transcriptional control element is a RAG1 transcriptional control element, a RAG2 transcriptional control element, an immunoglobulin heavy chain transcriptional control element, an immunoglobulin κ light chain transcriptional control element and/or an immunoglobulin λ light chain transcriptional control element. In some aspects, the transcriptional control element is of endogenous species origin. In some aspects, the transcriptional control element is a mouse transcriptional control element, a rat transcriptional control element or a human transcriptional control element. In some aspects, the transcriptional control element is an endogenous transcriptional control element (*e.g.,* the nucleotide sequence encoding the exogenous TdT is inserted into the non-human animal's genome at a position such that expression of the exogenous TdT is at least partially controlled by an endogenous transcriptional control element). In some aspects, transcriptional control elements may include those regulating transcription of: RAG1, RAG2, λ5, VpreB, CD34, CD45, AA4.1, CD45R, IL-7R, MHC class II, CD10, CD19, CD38, CD20, CD40, various immunoglobulin light and heavy chain V gene segments promoters and enhancers (see. *e.g.,* a list of various V gene segments listed on the International Immunogenetics Information System® website -- IMGT, imgt.org, *e.g.*, mouse V_H1-72 promoter and others, *etc.*). Transcriptional control elements may include those of human, mouse, rat, or other species origin.

[0039] In some aspects, the transcriptional control element induces expression of the encoded

TdT during T cell development. In some aspects, the transcriptional control element induces expression of TdT in CD4/CD8 double-negative (DN) thymocytes and/or CD4/CD8 double-positive (DP) thymocytes. In some aspects, the transcriptional control element is a transcriptional control element (*e.g.,* a promoter and/or enhancer) of a gene expressed during T cell development, in DN thymocytes and/or in DP thymocytes. In some aspects, the transcriptional control element is a RAG1 transcriptional control element, a RAG2 transcriptional control element, a TCRα transcriptional control element, a TCRβ transcriptional control element, a TCRγ transcriptional control element and/or a TCRδ transcriptional control element. In some aspects, the transcriptional control element is of endogenous species origin. In some aspects, the transcriptional control element is a mouse transcriptional control element, a rat transcriptional control element or a human transcriptional control element. In some aspects, the transcriptional control element is an endogenous transcriptional control element (*e.g.,* the nucleotide sequence encoding the exogenous TdT is inserted into the non-human animal's genome at a position such that expression of the exogenous TdT is at least partially controlled by an endogenous transcriptional control element). In some aspects, transcriptional control elements may include those regulating transcription of: RAG1, RAG2, Lck, ZAP-70, CD34, CD2, HSA, CD44, CD25, PTα, CD4, CD8, CD69, various TCRα, TCRβ, TCRδ, and TCRγ V gene segments promoters and enhancers (see. *e.g.,* a list of various V gene segments listed on the International Immunogenetics Information System® website -- IMGT, imgt.org, *etc.*) Transcriptional control elements may include those of human, mouse, rat, or other species origin.

[0040] In some aspects, the nucleic acid encoding the TdT is located in the genome of the rodent at or proximal to (*e.g.*, within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 kb) a genomic locus of a gene that is expressed during B cell development, in pro-B cells and/or in pre-B cells. In some embodiments, the nucleic acid sequence encoding TdT is located at or proximal to an immunoglobulin κ light chain locus, an immunoglobulin λ light chain locus, an immunoglobulin heavy chain locus, a RAG1 locus or a RAG2 locus.

[0041] In some aspects, the nucleic acid encoding the TdT is located in the genome of the rodent at or proximal to (*e.g.*, within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 kb) a genomic locus of a gene that is expressed during T cell development, in DN thymocytes and/or in DP thymocytes. In some aspects, the nucleic acid sequence encoding TdT is located at or proximal to a TCRα chain locus, a TCRβ chain locus, a TCRγ chain locus, a TCRδ chain locus, a RAG1 locus or a RAG2 locus.

[0042] In some aspects, the rodent described herein expresses elevated levels of TdT expression during one or more stages to T cell and/or B cell development (*e.g.*, in pro-B cells, in pre-B cells, in DN thymocytes and/or in DP thymocytes) compared to a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the genetically modified rodents described herein express at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% 150%, 200%, 250%, 300%, 350%, 400%, 450% or 500% more TdT in one or more stages of T cell and/or B cell development than a corresponding rodent.

[0043] In all the following aspects, the non-human animal is a rodent. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin κ chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-J immunoglobulin κ chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin κ chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin κ chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin κ chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin κ chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-J immunoglobulin κ chain junctions in the animal comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique immunoglobulin κ chain CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%. In some aspects, the non-human animal described herein has at least 900, 1000, 1100, 1200, 1300, 1400, 1500 or 1700 unique immunoglobulin κ chain CDR3 sequences per 10,000 immunoglobulin κ chain CDR3 sequences.

[0044] In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin λ chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-J immunoglobulin λ chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin λ chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin λ chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin λ chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin λ chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-J immunoglobulin λ chain junctions in the animal comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique immunoglobulin λ chain CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%. In some aspects, the non-human animal described herein has at least 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290 or 300 unique immunoglobulin λ chain CDR3 sequences per 10,000 immunoglobulin λ chain CDR3 sequences.

[0045] In some aspects, the non-human animal described herein has a greater percentage of V-D immunoglobulin heavy chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D immunoglobulin heavy chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-D immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-D immunoglobulin heavy chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D immunoglobulin heavy chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-D immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D immunoglobulin heavy chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D immunoglobulin heavy chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-D immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D immunoglobulin heavy chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D immunoglobulin heavy chain junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D immunoglobulin heavy chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D immunoglobulin heavy chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D immunoglobulin heavy chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D immunoglobulin heavy chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-D immunoglobulin heavy chain junctions in the animal comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique immunoglobulin heavy chain CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%.

[0046] In some aspects, the non-human animal described herein has a greater percentage of D-J immunoglobulin heavy chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J immunoglobulin heavy chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of D-J immunoglobulin heavy chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J immunoglobulin heavy chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of D-J immunoglobulin heavy chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J immunoglobulin heavy chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of D-J immunoglobulin heavy chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J immunoglobulin heavy chain junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of D-J immunoglobulin heavy chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J immunoglobulin heavy chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of D-J immunoglobulin heavy chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J immunoglobulin heavy chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the D-J immunoglobulin heavy chain junctions in the animal comprise non-template additions.

[0047] In some aspects, the non-human animal described herein has a greater percentage of V-J TCRα chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRα chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRα chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-J TCRα chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRα chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-J TCRα chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRα chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRα chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-J TCRα chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRα chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRα chain junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRα chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRα chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRα chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRα chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRα chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRα chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRα chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-J TCRα chain junctions in the animal comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique TCRα CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%.

[0048] In some aspects, the non-human animal described herein has a greater percentage of V-D TCRβ chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D TCRβ chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-D TCRβ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-D TCRβ chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D TCRβ chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-D TCRβ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D TCRβ chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D TCRβ chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-D TCRβ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D TCRβ chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D TCRβ chain junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D TCRβ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D TCRβ chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D TCRβ chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D TCRβ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D TCRβ chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D TCRβ chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D TCRβ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-D TCRβ chain junctions in the animal comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique TCRβ CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%.

[0049] In some aspects, the non-human animal described herein has a greater percentage of D-J TCRβ chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J TCRβ chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of D-J TCRβ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of D-J TCRβ chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J TCRβ chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of D-J TCRβ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of D-J TCRβ chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J TCRβ chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of D-J TCRβ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of D-J TCRβ chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J TCRβ chain junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of D-J TCRβ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of D-J TCRβ chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J TCRβ chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of D-J TCRβ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of D-J TCRβ chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J TCRβ chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of D-J TCRβ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the D-J TCRβ chain junctions in the animal comprise non-template additions.

[0050] In some aspects, the non-human animal described herein has a greater percentage of V-J TCRγ chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRγ chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRγ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-J TCRγ chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRγ chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-J TCRγ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRγ chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRγ chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-J TCRγ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRγ chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRγ chain junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRγ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRγ chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRγ chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRγ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRγ chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRγ chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRγ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-J TCRγ chain junctions in the animal comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique TCRγ CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%.

[0051] In some aspects, the non-human animal described herein has a greater percentage of V-D TCRδ chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D TCRδ chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-D TCRδ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-D TCRδ chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D TCRδ chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-D TCRδ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D TCRδ chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D TCRδ chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-D TCRδ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D TCRδ chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D TCRδ chain junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D TCRδ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D TCRδ chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D TCRδ chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D TCRδ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D TCRδ chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D TCRδ chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D TCRδ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-D TCRδ chain junctions in the animal comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique TCRδ CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%.

[0052] In some aspects, the non-human animal described herein has a greater percentage of D-J TCRδ chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J TCRδ chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of D-J TCRδ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of D-J TCRδ chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J TCRδ chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of D-J TCRδ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of D-J TCRδ chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J TCRδ chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of D-J TCRδ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of D-J TCRδ chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J TCRδ chain junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of D-J TCRδ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of D-J TCRδ chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J TCRδ chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of D-J TCRδ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of D-J TCRδ chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of D-J TCRδ chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of D-J TCRδ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the D-J TCRδ chain junctions in the animal comprise non-template additions.

[0053] In some aspects, the endogenous TdT loci in the non-human organism is intact. In some aspects the endogenous TdT loci is inactivated. For example, in some aspects, the endogenous TdT loci is deleted in whole or in part such that the non-human organism does not express endogenous TdT.

Rodents Expressing Human Variable Domain Antibodies and Exogenous TdT

[0054] In certain aspects, the genetically modified non-human animals and non-human animal ES cells comprising exogenous TdT as described herein also comprise in their germline and/or genome an immunoglobulin locus (exogenous or endogenous) containing an immunoglobulin variable region comprising unrearranged human immunoglobulin variable region gene segments and an immunoglobulin constant region comprising an immunoglobulin constant region gene and in which the unrearranged human immunoglobulin variable region gene segments are operably linked to the immunoglobulin constant region gene. In some aspects, the non-human animals and non-human ES cells comprise in their germline and/or genome multiple such immunoglobulin loci. For example, in some aspects, the genetically modified non-human animals and non-human animal ES cells comprise in their germline and/or genome at least one immunoglobulin locus comprising unrearranged human heavy chain variable region gene segments and at least one immunoglobulin locus comprising unrearranged human light chain variable region gene segments (*e.g.*, κ chain gene segments and/or λ chain gene segments). In some aspects, the genetically modified non-human animals and non-human animal ES cells comprise in their germline and/or genome at least one immunoglobulin locus comprising unrearranged human heavy chain variable region gene segments, at least one immunoglobulin locus comprising unrearranged human κ chain variable region gene segments and at least one immunoglobulin locus comprising unrearranged human λ chain variable region gene segments. In some aspects, genetically modified non-human animals, *e.g.*, genetically modified mice or rats, comprise in their germline and/or genome genetically modified immunoglobulin loci (genetically modified rearranged or unrearranged immunoglobulin loci) such that the mice make human, humanized, partially human, reverse chimeric (human variable and non-human constant regions) antibodies.

[0055] Immunoglobulin loci comprising human variable region gene segments are known in the art and can be found, for example, in U.S. Pat. Nos. 5,633,425, 5,770,429, 5,814,318, 6,075,181, 6,114,598, 6,150,584, 6,998,514, 7,795,494, 7,910,798, 8,232,449, 8,502,018, 8,697,940, 8,703,485, 8,754,287, 8,791,323, 8,907,157, 9,035,128, 9,145,588, and 9,206,263, as well as in U.S. Pat. Pub. Nos. 2008/0098490, 2010/0146647, 2011/0195454, 2012/0167237, 2013/0145484, 2013/0167256, 2013/0219535, 2013/0326647, 2013/0096287, 2014/013275, 2014/093908, and 2015/0113668, and in PCT Pub. Nos. WO2007117410, WO2008151081, WO2009157771, WO2010039900, WO2011004192, WO2011123708 and WO2014093908.

[0056] In some aspects, the human unrearranged immunoglobulin variable region gene segments are heavy chain gene segments and the immunoglobulin constant region gene is a heavy chain constant region gene. In some aspects, the human unrearranged immunoglobulin variable region gene segments are light chain, *e.g.,* κ chain, gene segments, and the immunoglobulin constant region gene is a heavy chain constant region gene.

[0057] In some aspects, the human unrearranged immunoglobulin variable region gene segments are heavy chain gene segments and the immunoglobulin constant region gene is a κ chain constant region gene. In some aspects, the human unrearranged immunoglobulin variable region gene segments are κ chain gene segments and the immunoglobulin constant region gene is a κ chain constant region gene. In some aspects, the human unrearranged immunoglobulin variable region gene segments are λ chain gene segments and the immunoglobulin constant region gene is a κ chain constant region gene. In some aspects, the human unrearranged immunoglobulin variable region gene segments are λ chain gene segments and the immunoglobulin constant region gene is a λ chain constant region gene.

[0058] In certain aspects, the immunoglobulin variable region contains unrearranged human Ig heavy chain variable region gene segments. In some aspects, the unrearranged human Ig variable region gene segments comprise a plurality of human V_H segments, one or more human D_H segments and one or more human J_H segments. In some aspects, the unrearranged human Ig variable region gene segments comprise at least 3 V_H gene segments, at least 18 V_H gene segments, at least 20 V_H gene segments, at least 30 V_H gene segments, at least 40 V_H gene segments, at least 50 V_H gene segments, at least 60 V_H gene segments, at least 70 V_H gene segments, or at least 80 V_H gene segments. In some aspects, the unrearranged human Ig gene segments include all of the human D_H gene segments. In some aspects, the unrearranged human Ig gene segments include all of the human J_H gene segments. Exemplary variable regions comprising Ig heavy chain gene segments are provided, for example, in Macdonald et al., Proc. Natl. Acad. Sci. USA 111:5147-52 and supplemental information. In some aspects, the non-human animals described herein have a restricted immunoglobulin heavy chain locus characterized by a single polymorphic human V_H gene segment, a plurality of D_H gene segments and a plurality of J_H gene segments (*e.g.*, as described in U.S. Pat. Pub. No. 2013/0096287). In some aspects the V_H gene segment is VH1-2 or VH1-69.

[0059] In various aspects, the immunoglobulin locus modifications described herein do not affect fertility of the non-human animal. In some aspects, the heavy chain locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both. In some aspects, the genome of the genetically modified non-human animal comprises an ectopically located Adam6a gene, Adam6b gene, or both. Exemplary non-human animals expressing exogenous Adam6a and/or Adam6b are described in U.S. Pat. Nos. 8,642,835 and 8,697,940.

[0060] In some aspects, the human immunoglobulin heavy chain variable region gene segments rearrange during B cell development to generate rearranged human heavy chain variable region genes in the B cells of the non-human organism. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-D immunoglobulin heavy chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J immunoglobulin heavy chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-D and/or D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-D and/or D-J immunoglobulin heavy chain junctions in the animal comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique immunoglobulin heavy chain CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%.

[0061] In certain aspects, the immunoglobulin variable region contains unrearranged human Ig κ variable region gene segments. In some aspects, the unrearranged human immunoglobulin variable region gene segments comprise a plurality of human V_K segments and one or more human J_K segments. In some aspects, the unrearranged human immunoglobulin variable region gene segments comprise all of the human Jκ segments. In some aspects, the immunoglobulin variable region gene segments comprise four functional V_K segments and all human J_K segments. In some aspects, the immunoglobulin variable region gene segments comprise 16 functional V_K segments and all human J_K segments (*e.g.*, all functional human Vκ segments and J_K segments). In some aspects, the unrearranged human immunoglobulin variable region gene segments comprise all of the human Vκ segments and all human J_K segments. Exemplary variable regions comprising Ig κ gene segments are provided, for example, in Macdonald *et al., Proc. Natl. Acad. Sci. USA* 111:5147-52 and supplemental information. In some aspects, the non-human animals provided herein have a restricted immunoglobulin light chain locus characterized by no more than two human V_L gene segments and a plurality of J_L gene segments (*e.g.*, dual light chain mice, or DLC, as described in U.S. Pat. Pub. No. 2013/0198880). In some aspects the V_L gene segments are $V_κ$ gene segments. In some aspects the V_L gene segments are $V_λ$ gene segments. In some aspects the $V_κ$ gene segments are IGKV3-20 and IGKV1-39.

[0062] In some aspects, the human immunoglobulin κ variable region gene segments rearrange during B cell development to generate rearranged human κ variable region genes in the B cells of the non-human organism. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin κ chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some embodiments, the non-human animal described herein has a lower percentage of V-J immunoglobulin κ chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin κ chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin κ chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions containing at least 2 N-additions in the genetically modified non-human animals provided herein is greater than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin κ chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin κ chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-J immunoglobulin κ chain junctions in the animal comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique immunoglobulin κ chain CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%. In some aspects, the non-human animal described herein has at least 900, 1000, 1100, 1200, 1300, 1400, 1500 or 1700 unique immunoglobulin κ chain CDR3 sequences per 10,000 immunoglobulin κ chain CDR3 sequences.

[0063] In certain aspects, the immunoglobulin variable region contains unrearranged human Ig λ variable region gene segments. In some aspects, the unrearranged human immunoglobulin variable region gene segments comprise a plurality of human V_λ segments and one or more human J_λ segments. In some aspects, the unrearranged human immunoglobulin variable region gene segments comprise all of the human V_λ segments. In some aspects, the unrearranged human immunoglobulin variable region gene segments comprise all of the human J_λ segments. Exemplary variable regions comprising Ig λ gene segments are provided, for example, U.S. Pat. Pub. Nos. 2012/0073004 and 2002/0088016.

[0064] In some aspects, the human immunoglobulin λ variable region gene segments rearrange during B cell development to generate rearranged human λ variable region genes in the B cells of the non-human organism. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin λ chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-J immunoglobulin λ chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin λ chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin λ chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some embodiments, the non-human animal described herein has a greater percentage of V-J immunoglobulin λ chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin λ chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-J immunoglobulin λ chain junctions in the animal may comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique immunoglobulin λ chain CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%. In some aspects, the non-human animal described herein has at least 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290 or 300 unique immunoglobulin λ chain CDR3 sequences per 10,000 immunoglobulin λ chain CDR3 sequences.

[0065] In some aspects, the immunoglobulin variable region comprising unrearranged human immunoglobulin variable region gene segments also include human immunoglobulin variable region intergenic sequences. In some aspects, the immunoglobulin variable region includes non-human (*e.g.*, rodent, rat, mouse) Ig variable region intergenic sequences. In some aspects, the intergenic sequence is of endogenous species origin.

[0066] In some aspects, the non-human organism comprises in its germline and/or genome an immunoglobulin locus comprising a rearranged heavy chain variable region (a universal heavy chain variable region). In some aspects, the rearranged Ig heavy chain variable region gene is a human rearranged Ig heavy chain variable region gene. Exemplary rearranged Ig heavy chain variable regions are provided in U.S. Patent Pub. No. 2014/0245468. In some aspects, the non-human organism comprising a universal heavy chain variable region is used to produce bispecific antibodies.

[0067] In some aspects, the non-human organism comprises in its germline and/or genome an immunoglobulin locus comprising a rearranged light variable region (a universal light chain variable region). In some aspects, the rearranged Ig light chain variable region gene is a human rearranged Ig light chain variable region gene. Exemplary rearranged Ig light chain variable regions are provided in, *e.g.,* U.S. Patent Pub. Nos. 2011/0195454, 2012/0021409, 2012/0192300, 2013/0045492, 2013/0185821, 2013/0302836, 2015/0313193, 2015/0059009, and 2013/0198879. In some aspects, the non-human organism ("universal light chain" organism) comprising a universal light chain variable region is used to produce bispecific antibodies.

[0068] In some aspects, the non-human organism comprise in its germline and/or genome a light chain immunoglobulin locus comprising a limited repertoire of light chain variable gene segments (*e.g.*, a dual light chain variable region comprising two light chain variable gene segments). In some aspects, the light chain variable gene segments in the limited repertoire of light chain gene segments are a human light chain gene segments. Exemplary dual light chain variable regions are provided in U.S. Patent Pub. No. 2013/0198880. In some aspects, the non-human organism comprising a dual light chain variable region is used to produce bispecific antibodies.

[0069] In yet other aspects, the non-human organism may comprise in its germline and/or genome a light chain and/or a heavy chain immunoglobulin locus that includes insertions and/or replacements of histidine codons designed to introduce pH-dependent binding properties to the antibodies generated in such non-human organism. In some of such aspects, the histidine codons are inserted and/or replaced in the nucleic acid sequences encoding CDR3. Various such light and/or heavy immunoglobulin loci are provided in U.S. Patent Nos. 9,301,510, 9,334,334, U.S. Patent Application Publication Nos. 2013/0247236, 20140013456.

[0070] In some aspects, the immunoglobulin constant region comprises a heavy chain constant region gene. In some aspects, the heavy chain constant region gene is a human heavy chain constant region gene. In some aspects, the heavy chain constant region gene is of endogenous species origin. In some aspects, the heavy chain constant region gene is a mouse constant region gene or a rat constant region gene. In some aspects, the constant region gene is a mixture of human and non-human sequence. For example, in some aspects, the constant region gene encodes a human CH1 region and a non-human (*e.g.*, endogenous species origin, mouse, rat) CH2 and/or CH3 region. In some aspects, the heavy chain constant region gene is an Cμ, Cδ, Cγ (Cγ1, Cγ2, Cγ3, Cγ4), Cα or Cε constant region gene. In some aspects, the constant region gene is an endogenous constant region gene. In some aspects, the constant region gene encodes a mutated CH1 region so that the non-human animal expresses heavy chain only antibodies (see., *e.g.,* U.S. Patent No. 8,754,287, U.S. Patent Application Publication No. 2015/0289489). In some aspects, *e.g.*, where the goal is to generate heavy chains to make bispecific antibodies (*e.g.*, in universal or dual light chain organisms), the Fc domains of the heavy chains comprise modifications to facilitate heavy chain heterodimer formation and/or to inhibit heavy chain homodimer formation. Such modifications are provided, for example, in U.S. Pat. Nos. 5,731,168, 5,807,706, 5,821,333, 7,642,228 and 8,679,785 and in U.S. Pat. Pub. No. 2013/0195849.

[0071] In some aspects, the immunoglobulin constant region comprises a light chain constant region gene. In some aspects, the light chain constant region gene is a κ constant region gene. In some aspects, the light chain constant region gene is a λ constant region gene. In some aspects, the light chain constant region gene is of endogenous species origin. In some aspects, the light chain constant region gene is a mouse constant region gene or a rat constant region gene. In some aspects, the light chain constant region gene is a mixture of human and non-human sequence.

[0072] In some aspects, the immunoglobulin variable region comprising human variable region gene segments and the immunoglobulin constant region gene to which the variable region gene segments are operably linked are located at an endogenous immunoglobulin locus. In some aspects, the endogenous immunoglobulin locus is an endogenous heavy chain locus. In some aspects, the endogenous immunoglobulin locus is an endogenous κ locus. In some aspects, the endogenous immunoglobulin locus is an endogenous λ locus. In some aspects, the constant region gene to which the human variable region gene segments are operably linked is an endogenous constant region gene.

[0073] In some aspects, one or more of the endogenous immunoglobulin loci or a portion of the one or more endogenous loci (*e.g.,* a variable region and/or a constant region) in the genome of the non-human animal provided herein is inactivated. Endogenous immunoglobulin variable region gene loci and portions thereof can be inactivated using any method known in the art, including, but not limited to, the deletion of the locus or a portion thereof from the genome of the organism, the replacement of a locus or a portion thereof with a different nucleic acid sequence, the inversion of a portion of the locus and/or the displacement of a portion of the locus to another position in the genome of the non-human organism. In some aspects the inactivation of the locus is only a partial inactivation. In some aspects, the variable region of the locus is inactivated but the constant region remains functional (*e.g.*, because it is operably linked to non-endogenous variable region gene segments).

[0074] In some aspects, the genetically modified non-human animal includes an inactivated endogenous immunoglobulin heavy chain locus. In some aspects, the endogenous immunoglobulin heavy chain locus or a portion thereof is inactivated by deletion, replacement, displacement and/or inversion of at least part of the endogenous variable region of the endogenous heavy chain locus. In some aspects, the at least part of the variable region of the endogenous heavy chain locus that is deleted, replaced, displaced, and/or inverted comprises the J segments of the variable region. In some aspects, the endogenous immunoglobulin heavy chain locus or portion thereof is inactivated by deletion, replacement, displacement and/or inversion of at least part of the endogenous constant region of the endogenous heavy chain locus. In some aspects, the at least part of the constant region of the endogenous heavy chain locus that is deleted, replaced, displaced, and/or inverted may comprise the Cμ gene of the endogenous constant region.

[0075] In some aspects, the genetically modified non-human animal includes an inactivated endogenous immunoglobulin κ chain locus. In some aspects, the endogenous immunoglobulin κ chain locus or a portion thereof is inactivated by deletion, replacement, displacement and/or inversion of at least part of the endogenous variable region of the endogenous κ chain locus. In some aspects, the at least part of the variable region of the endogenous κ chain locus that is deleted, replaced, displaced, and/or inverted comprises the J segments of the variable region. In some aspects, the endogenous immunoglobulin κ chain locus or portion thereof is inactivated by deletion, replacement, displacement and/or inversion of at least part of the endogenous constant region of the endogenous κ chain locus. In some aspects, the at least part of the constant region of the endogenous κ chain locus that is deleted, replaced, displaced, and/or inverted comprises the Cκ gene of the endogenous constant region.

[0076] In some aspects, the genetically modified non-human animal includes an inactivated endogenous immunoglobulin λ chain locus. In some aspects, the endogenous immunoglobulin λ chain locus or a portion thereof is inactivated by deletion, replacement, displacement and/or inversion of at least part of an endogenous variable region of the endogenous λ chain locus. In some aspects, the at least part of at least one V-J-C gene cluster in the endogenous λ chain locus is deleted, replaced, displaced, and/or inverted. In some aspects, the endogenous immunoglobulin λ chain locus or portion thereof is inactivated by deletion, replacement, displacement and/or inversion of at least part of an endogenous constant region of the endogenous λ chain locus. In some aspects, the at least part of the constant region of the endogenous λ chain locus that is deleted, replaced, displaced, and/or inverted comprises a Cλ gene of the endogenous constant region.

[0077] In some aspects, the genetically modified non-human animal provided herein expresses antibodies having human variable domains (*e.g.*, a human variable domain derived from the unrearranged human variable region gene segments described herein). In some aspects, the human variable domain is a human heavy chain variable domain. In some aspects, the antibodies are heavy chain only antibodies. In some aspects, the human variable domain is a human light chain variable domain. In some aspects, the antibodies produced by the non-human animals have both human heavy chain variable domains and human light chain variable domains. In some aspects, the antibodies have human heavy chain constant domains. In some aspects, the antibodies have human light chain constant domains. In some aspects, the heavy and/or light chain constant domain is of non-human origin. For example, in some aspects, the heavy chain constant domain is of endogenous species origin. In some aspects, the heavy chain constant domain is of mouse or rat origin. In some aspects, the light chain constant domain is of endogenous species origin. In some aspects, the light chain constant domain is of rat or mouse origin.

Rodents expressing human variable domain T cell receptors and exogenous TdT

[0078] In certain aspects, the genetically modified non-human animals and non-human animal ES cells that comprise exogenous TdT as described herein also comprise in their germline and/or genome a TCR locus (exogenous or endogenous) containing a TCR variable region comprising unrearranged human TCR variable region gene segments and a TCR constant region comprising a TCR constant region gene and in which the unrearranged human TCR variable region gene segments are operably linked to the TCR constant region gene. In some aspects, various genetically modified non-human animals, *e.g.*, genetically modified mice, comprise in their germline and/or genome genetically modified T cell receptor loci (genetically modified TCRα, β, γ and/or δ loci) such that the mice express human, humanized, partially human, reverse chimeric (human variable and non-human constant regions) T cell receptors. In one aspect, the exemplary non-human animal is provided in U.S. Patent No. 9,113,616 and International Pub. No. WO 2016/164492.

[0079] In some aspects, the TCR constant region gene is a non-human TCR constant region gene. In some aspects, the TCR constant region gene is a rodent constant region gene, such as a rat constant region gene or a mouse constant region gene. In some aspects, the constant region gene is of endogenous species origin. In some aspects, the TCR constant region gene is a human constant region gene.

[0080] In some aspects, the non-human animals and non-human ES cells comprise in their germline and/or genome multiple such TCR loci. For example, in some aspects, the genetically modified non-human animals and non-human animal ES cells may comprise in their germline and/or genome at least one TCR locus comprising unrearranged TCRα variable region gene segments and at least one TCR locus comprising unrearranged TCRβ variable region gene segments. In some aspects, the genetically modified non-human animals and non-human animal ES cells may comprise in their germline and/or genome at least one TCR locus comprising unrearranged human TCRγ variable region gene segments and at least one TCR locus comprising unrearranged human TCRδ variable region gene segments.

[0081] In some aspects, the human unrearranged TCR variable region gene segments are TCRα gene segments and the TCR constant region gene is a TCRα constant region gene. In some aspects, the human unrearranged TCR variable region gene segments are TCRβ chain gene segments and the TCR constant region gene is a TCRβ constant region gene. In some aspects, the human unrearranged TCR variable region gene segments are TCRγ chain gene segments and the TCR constant region gene is a TCRγ constant region gene. In some aspects, the human unrearranged TCR variable region gene segments are TCRδ chain gene segments and the TCR constant region gene is a TCRδ constant region gene. Exemplary variable regions comprising human TCR gene segments are provided, for example, in U.S. Patent No. 9,113,616 and Li et al., Nature Medicine 16:1029-1035 (2010).

[0082] In some aspects, the TCR variable region contains unrearranged human TCRβ variable region gene segments. In some aspects, the human TCRβ variable region gene segments rearrange during T cell development to generate rearranged human TCRβ variable region genes in the T cells of the non-human organism. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J TCRβ junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J TCRβ junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J TCRβ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-D TCRβ junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J TCRβ junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-D and/or D-J TCRβ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J TCRβ junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J TCRβ junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J TCRβ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J TCRβ junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J TCRβ junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J TCRβ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J TCRβ junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J TCRβ junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J TCRβ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal provided herein has a greater percentage of V-D and/or D-J TCRβ junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J TCRβ junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J TCRβ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-D and/or D-J TCRβ junctions in the animal comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique TCRβ CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%.

[0083] In some aspects, the TCR variable region contains unrearranged human TCRα variable region gene segments. In some aspects, the human TCRα variable region gene segments rearrange during T cell development to generate rearranged human TCRα variable region genes in the T cells of the non-human organism. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRα junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRα junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRα junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-D TCRα junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRα junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-J TCRα junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRα junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRα junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-J TCRα junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRα junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRα junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRα junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRα junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRα junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRα junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRα junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRα junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRα junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-J TCRα junctions in the animal comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique TCRα CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%.

[0084] In some aspects, the TCR variable region may contain unrearranged human TCRδ variable region gene segments. In some aspects, the human TCRδ variable region gene segments rearrange during T cell development to generate rearranged human TCRδ variable region genes in the T cells of the non-human organism. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J TCRδ junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J TCRδ junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J TCRδ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-D TCRδ junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J TCRδ junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-D and/or D-J TCRδ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J TCRδ junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J TCRδ junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J TCRδ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J TCRδ junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J TCRδ junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J TCRδ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J TCRδ junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J TCRδ junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J TCRδ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J TCRδ junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J TCRδ junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J TCRδ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-D and/or D-J TCRδ junctions in the animal comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique TCRδ CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%.

[0085] In some aspects, the TCR variable region contain unrearranged human TCRγ variable region gene segments. In some aspects, the human TCRγ variable region gene segments rearrange during T cell development to generate rearranged human TCRγ variable region genes in the T cells of the non-human organism. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRγ junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRγ junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRγ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-D TCRγ junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRγ junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-J TCRγ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some embodiments, the non-human animal described herein has a greater percentage of V-J TCRγ junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRγ junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-J TCRγ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRγ junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRγ junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRγ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRγ junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRγ junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRγ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J TCRγ junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J TCRγ junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J TCRγ junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-J TCRγ junctions in the animal comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique TCRγ CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%.

[0086] In some aspects, the TCR variable region comprising unrearranged human TCR variable region gene segments also include human TCR variable region intergenic sequences. In some aspects, the TCR variable region include non-human (*e.g.*, rodent, rat, mouse) TCR variable region intergenic sequences. In some aspects, the intergenic sequences are of endogenous species origin.

[0087] In some aspects, TCR variable region comprising human variable region gene segments and the TCR constant region gene to which the variable region gene segments are operably linked are located at an endogenous TCR locus. In some aspects, the endogenous TCR locus is an endogenous TCRα locus. In some aspects, the endogenous TCR locus is an endogenous TCRβ locus. In some aspects, the endogenous TCR locus is an endogenous TCRγ locus. In some aspects, the endogenous TCR locus is an endogenous TCRδ locus. In some aspects, the constant region gene to which the human variable region gene segments are operably linked is an endogenous constant region gene, *e.g.,* the corresponding endogenous constant region.

[0088] In some aspects, one or more of the endogenous TCR loci or a portion of the one or more endogenous loci (*e.g.,* a variable region and/or a constant region) in the genome of the non-human animal described herein is inactivated. Endogenous TCR variable region gene loci and portions thereof can be inactivated using any method known in the art, including, but not limited to, the deletion of the locus or a portion thereof from the genome of the organism, the replacement of a locus or a portion thereof with a different nucleic acid sequence, the inversion of a portion of the locus and/or the displacement of a portion of the locus to another position in the genome of the non-human organism. In some aspects the inactivation of the locus is only a partial inactivation. In some aspects, the variable region of the locus is inactivated but the constant region remains functional (*e.g.*, because it is operably linked to non-endogenous variable region gene segments). Examples of inactivated TCR loci are described, for example, in Mombaerts et al., Proc. Natl. Acad. Sci. USA 88:3084-3087 (1991) and Mombaerts et al., Nature 390:225-231 (1992).

[0089] In some aspects, the genetically modified non-human animal described herein expresses TCR having human variable domains (*e.g.*, a human variable domain derived from the unrearranged human variable region gene segments described herein). In some aspects, the human variable domain is a human TCRα variable domain. In some aspects, the human variable domain may be a human TCRβ variable domain. In some aspects, the human variable domain is a human TCRγ variable domain. In some aspects, the human variable domain is a human TCRδ variable domain. In some aspects, the TCR produced by the non-human animals may have both human TCRα variable domains and human TCRβ variable domains. In some aspects, the TCR produced by the non-human animals have both human TCRγ variable domains and human TCRδ variable domains. In some aspects, the TCR produced by the non-human animals have both human TCRα variable domains and human TCRβ variable domains, and both human TCRγ variable domains and human TCRδ variable domains. In some aspects, the TCR have human constant domains. In some aspects, the constant domains are of non-human origin. For example, in some aspects, constant domains are of endogenous species origin. In some aspects, the constant domains are of mouse or rat origin.

Rodents expressing chimeric antigen receptors (CARs) and exogenous TdT

[0090] In certain aspects, described herein are genetically modified non-human animals and non-human animal ES cells comprising exogenous TdT as described herein that also comprise chimeric antigen receptor (CAR) loci. Such CAR loci generally comprise a variable region and a constant region. The variable region includes unrearranged human Ig variable region gene segments, while the constant region locus includes a TCR constant region gene, wherein the Ig variable region gene segments are operably linked to the constant region gene. In some aspects, the TCR constant region gene is a non-human TCR constant region gene. In some aspects, the TCR constant region gene is a rodent constant region gene, such as a rat constant region gene or a mouse constant region gene. In some aspects, the constant region gene is of endogenous species origin. In some aspects, the TCR constant region gene is a human constant region gene.

[0091] In some aspects, the CAR loci described herein are located at an endogenous TCR loci. For example, in some aspects, a CAR locus comprising a TCRα constant region gene is located at an endogenous TCRα constant region locus. In some aspects, such a locus is created by replacing some or all of the TCRα unrearranged variable region with an unrearranged Ig variable region. In some aspects, a CAR locus comprising a TCRβ constant region gene is located at an endogenous TCRβ constant region locus. In some aspects, such a locus is created by replacing some or all of the TCRβ unrearranged variable region with an unrearranged Ig variable region.

[0092] In certain aspects, the CAR variable region locus will contain unrearranged human Ig variable region gene segments. Exemplary variable region loci comprising human variable region gene segments have been described in the art. For example, such loci are described in U.S. Pat. Nos. 5,633,425, 5,770,429, 5,814,318, 6,075,181, 6,114,598, 6,150,584, 6,998,514, 7,795,494, 7,910,798, 8,232,449, 8,502,018, 8,697,940, 8,703,485, 8,754,287, 8,791,323, 8,907,157, 9,035,128, 9,145,588, and 9,206,263, as well as in U.S. Pat. Pub. Nos. 2008/0098490, 2010/0146647, 2011/0195454, 2012/0167237, 2013/0145484, 2013/0167256, 2013/0219535, 2013/0326647, 2014/013275, 2014/093908, 2015/0113668 and 2016/0081314, and in PCT Pub. Nos. WO2007117410, WO2008151081, WO2009157771, WO2010039900, WO2011004192, WO2011123708, WO2014093908 and WO2016/044745.

[0093] In certain aspects, the CAR variable region locus contain unrearranged human Ig heavy chain variable region gene segments. In some aspects, the unrearranged human Ig variable region gene segments comprise a plurality of human V_H segments, one or more human D_H segments and one or more human J_H segments. In some aspects, the unrearranged human Ig variable region gene segments comprise at least 3 V_H gene segments, at least 18 V_H gene segments, at least 20 V_H gene segments, at least 30 V_H gene segments, at least 40 V_H gene segments, at least 50 V_H gene segments, at least 60 V_H gene segments, at least 70 V_H gene segments, or at least 80 V_H gene segments. In some aspects, the unrearranged human Ig gene segments include all of the human D_H gene segments. In some aspects, the CAR variable region further comprises TCRβ variable region gene segments (*e.g.*, V, D and/or J gene segments). In one aspect, the CAR variable region further comprise distal TCR Vβ gene segments, *e.g.*, TCR Vβ31 gene segment. In another aspect, the distal TCR Vβ gene segments, *e.g.*, TCR Vβ31 gene segment, has been functionally inactivated or deleted. In some aspects, the unrearranged human Ig gene segments include all of the human J_H gene segments. Exemplary variable regions comprising Ig heavy chain gene segments are provided, for example, in Macdonald et al., Proc. Natl. Acad. Sci. USA 111:5147-52 and supplemental information.

[0094] In some aspects, the human immunoglobulin heavy chain variable region gene segments rearrange during T cell development to generate rearranged human heavy chain variable region genes in the T cells of the non-human organism. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-D immunoglobulin heavy chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J immunoglobulin heavy chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-D and/or D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some embodiments, the non-human animal described herein has a greater percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-D and/or D-J immunoglobulin heavy chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-D and/or D-J immunoglobulin heavy chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-D and/or D-J immunoglobulin heavy chain junctions in the animal may comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique immunoglobulin heavy chain CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%.

[0095] In some aspects, the CAR variable gene locus comprising unrearranged human Ig heavy chain variable region gene segments may also include human Ig heavy chain variable region intergenic sequences. In some aspects, the CAR variable gene locus includes non-human (e.g., rodent, rat, mouse) Ig heavy chain variable region intergenic sequences. In some aspects, the CAR variable gene locus includes human or non-human (*e.g.,* rodent, rat, mouse) TCRβ variable region intergenic sequences. For example, in some aspects the unrearranged variable region of the CAR locus comprises one or more (*e.g., 1,* 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20) trypsinogen (TRY) genes (*e.g.,* TRY genes and/or pseudogenes normally present in the TCRβ variable region locus). In some aspects, the TRY genes are non-human, *e.g*., mouse TRY genes. In some aspects, the mouse TRY genes are selected from the group consisting of Try1, Try2, Try3, Try4, Try5, Try6, Try7, Try8, Try9, Try10, Try11, Try12, Try13, Try14, Try15, Try16, Try17, Try18, Try19 and Try20. In some aspects, one or more TRY genes are located upstream of the V_H segments of the unrearranged variable region. In some aspects, one or more TRY genes are located downstream of the V_H segments and upstream of the D_H segments of the unrearranged variable region. In some aspects, Try1-7 are located upstream of the V_H segments of the unrearranged variable region and Try 8-20 are located downstream of the V_H segments and upstream of the D_H segments of the unrearranged variable region. Additional information regarding the TRY genes located in the human and/or mouse TCRβ locus is provided in Glusman et al., Immunity 15:337-349 (2001) and Skok et al., Nature Immunology 8:378-387 (2007). In some aspects, the CAR gene locus comprises non-human regulatory elements (*e.g*., non-human promoters and/or enhancers). In some aspects, the non-human regulatory elements are rodent regulatory elements (*e.g*., rat or mouse promoters or enhancers). In some aspects, the CAR locus comprises an IgM enhancer (Eμ). In some aspects, the IgM enhancer is a non-human Eμ (*e.g.,* a rodent Eμ, such as a mouse or rat Eμ).

[0096] In certain aspects, the CAR variable region locus contains unrearranged human Ig κ variable region gene segments. In some aspects, the unrearranged human immunoglobulin variable region gene segments comprise a plurality of human V_K segments and one or more human J_K segments. In some aspects, the immunoglobulin variable region gene segments comprise four functional V_K segments and all human J_K segments. In some aspects, the immunoglobulin variable region gene segments comprise 16 functional V_K segments and all human J_K segments. In some aspects, the unrearranged human immunoglobulin variable region gene segments comprise all of the human V_K segments and all human J_K segments (e.g., all functional human V_K segments and J_K segments). Exemplary variable regions comprising Ig κ gene segments are provided, for example, in Macdonald et al., Proc. Natl. Acad. Sci. USA 111:5147-52 and supplemental information. In some aspects, the unrearranged human immunoglobulin variable region gene segments comprise all of the human J_K segments. In some aspects, the CAR variable region further comprises TCRα variable region gene segments (*e.g*., V, and/or J gene segments).

[0097] In some aspects, the human immunoglobulin κ variable region gene segments rearrange during T cell development to generate rearranged human κ variable region genes in the T cells of the non-human organism. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin κ chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-J immunoglobulin κ chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin κ chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin κ chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin κ chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin κ chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin κ chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin κ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-J immunoglobulin κ chain junctions in the animal comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique immunoglobulin κ chain CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%. In some aspects, the non-human animal described herein has at least 900, 1000, 1100, 1200, 1300, 1400, 1500 or 1700 unique immunoglobulin κ chain CDR3 sequences per 10,000 immunoglobulin κ chain CDR3 sequences.

[0098] In certain aspects, the CAR variable region locus contain unrearranged human Ig λ variable region gene segments. In some aspects, the unrearranged human immunoglobulin variable region gene segments comprise a plurality of human V_λ segments and one or more human J_λ segments. In some aspects, the unrearranged human immunoglobulin variable region gene segments comprise all of the human V_λ segments (*e.g.*, all functional human V_λ segments). In some aspects, the unrearranged human immunoglobulin variable region gene segments comprise all of the human J_λ segments. In some aspects, the CAR variable region further comprises TCRα variable region gene segments (*e.g.*, V, and/or J gene segments). Exemplary variable regions comprising Ig λ gene segments are provided, for example, U.S. Pat. Pub. Nos. 2012/0073004 and 2002/0088016.

[0099] In some aspects, the human immunoglobulin λ variable region gene segments rearrange during T cell development to generate rearranged human λ variable region genes in the T cells of the non-human organism. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin λ chain junctions containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions containing non-template additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a lower percentage of V-J immunoglobulin λ chain junctions not containing non-template additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions not containing non-template additions in the genetically modified non-human animals described herein is less than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin λ chain junctions containing at least 1 N-addition than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions containing at least 1 N-addition in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin λ chain junctions containing at least 2 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions containing at least 2 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin λ chain junctions containing at least 3 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions containing at least 3 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, the non-human animal described herein has a greater percentage of V-J immunoglobulin λ chain junctions containing at least 4 N-additions than a corresponding non-human animal that does not have a nucleic acid encoding an exogenous TdT in its genome. In some aspects, the percentage of V-J immunoglobulin λ chain junctions containing at least 4 N-additions in the genetically modified non-human animals described herein is greater than percentage of V-J immunoglobulin λ chain junctions in the corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30% or 40%. In some aspects, at least 10%, 15%, 20%, 25%, 30%, 35% or 40% of the V-J immunoglobulin λ chain junctions in the animal comprise non-template additions. In some aspects, the non-human animal has a greater frequency of unique immunoglobulin λ chain CDR3 sequences then a corresponding non-human animal by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100%. In some aspects, the non-human animal described herein has at least 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290 or 300 unique immunoglobulin λ chain CDR3 sequences per 10,000 immunoglobulin λ chain CDR3 sequences.

[0100] In some aspects, the CAR variable gene locus containing unrearranged human Ig light chain variable region gene segments also include human Ig light chain variable region intergenic sequences (*e.g.*, κ variable region intergenic sequences and/or λ variable region intergenic sequences). In some aspects, the CAR variable gene locus includes non-human (*e.g.,* rodent, rat, mouse) Ig light chain variable region intergenic sequences (*e.g.,* κ variable region intergenic sequences and/or λ variable region intergenic sequences). In some aspects, the CAR variable gene locus includes human or non-human (*e.g.*, rodent, rat, mouse) TCRα variable region intergenic sequences. In some aspects, the CAR gene locus comprises non-human regulatory elements (*e.g.*, non-human promoters and/or enhancers). In some aspects, the non-human regulatory elements are rodent regulatory elements (*e.g.*, rat or mouse promoters or enhancers).

[0101] In some aspects, the CAR variable region locus is a rearranged variable region locus comprising a Ig heavy chain variable region gene (a universal heavy chain variable region). In some aspects, the rearranged Ig heavy chain variable region gene is a human rearranged Ig heavy chain variable region gene. Use of universal heavy chain variable regions facilitate the generation of bispecific antibodies in which at least one antigen-binding domain has specificity for a peptide/MHC complex. Exemplary rearranged Ig heavy chain variable regions are provided in U.S. Patent Pub. No. 2014/0245468.

[0102] In some aspects, the CAR variable region locus is a rearranged variable region locus comprising a Ig light chain variable region gene (a universal light chain variable region). In some aspects, the rearranged Ig light chain variable region gene is a human rearranged Ig light chain variable region gene. Use of universal light chain variable regions facilitate the generation of bispecific antibodies in which at least one antigen-binding domain has binding specificity for a peptide/MHC complex. Exemplary rearranged Ig heavy chain variable regions are provided in U.S. Patent Pub. No. 2013/0185821.

Other Genetic Modifications

[0103] In some aspects, the genetically modified non-human animals and ES cells described herein that express exogenous TdT, humanized TCRs or CARs also express and/or comprise in their genome loci encoding humanized MHC class I α chain polypeptides (*e.g.*, humanized HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-g, HLA-K and/or HLA-L). In some aspects, the humanized MHC class I α chain polypeptide is fully human. In some aspects, the humanized MHC class I α chain polypeptide comprises a human extracellular domain (*e.g.,* a human α1, α2, and α3 domains) and a cytoplasmic domain of endogenous species origin. Humanized MHC class I α chain polypeptides, loci encoding humanized MHC class I α chain polypeptides and non-human animals expressing humanized MHC class I α chain polypeptides are described in U.S. Pat. Pub. Nos. 2013/0111617, 2013/0185819 and 2014/0245467.

[0104] In some aspects, the genetically modified non-human animals and ES cells described herein that express exogenous TdT, humanized TCRs or CARs also express and/or comprise in their genome a locus encoding humanized β-2-microglobulin polypeptide. Humanized β-2-microglobulin polypeptides, loci encoding humanized β-2-microglobulin polypeptides and non-human animals expressing humanized β-2-microglobulin polypeptides are described in U.S. Pat. Pub. Nos. 2013/0111617 and 2013/0185819.

[0105] In some aspects, the genetically modified non-human animals and ES cells described herein that express exogenous TdT, humanized TCRs or CARs may also express and/or comprise in their genome a loci encoding humanized MHC class II α chain polypeptides (*e.g.*, humanized HLA-DMA, HLA-DOA, HLA-DPA, HLA-DQA and/or HLA-DRA). In some aspects, the humanized MHC class II α chain polypeptide is fully human. In some aspects, the humanized MHC class II α chain polypeptide comprises a human extracellular domain and a cytoplasmic domain of endogenous species origin. Humanized MHC class II α chain polypeptides, loci encoding humanized MHC class II α chain polypeptides and non-human animals expressing humanized MHC class II α chain polypeptides are described in U.S. Patent Nos. 8,847,005 and

9,043,996 and U.S. Pat. Pub. No. 2014/0245467.

[0106] In some aspects, the genetically modified non-human animals and ES cells described herein that express exogenous TdT, humanized TCRs or CARs also express and/or comprise in their genome a loci encoding humanized MHC class II β chain polypeptides (*e.g.*, humanized HLA-DMB, HLA-DOB, HLA-DPB, HLA-DQB and/or HLA-DRB). In some aspects, the humanized MHC class II β chain polypeptide is fully human. In some aspects, the humanized MHC class II β chain polypeptide comprises a human extracellular domain and a cytoplasmic domain of endogenous species origin. Humanized MHC class II β chain polypeptides, loci encoding humanized MHC class II β chain polypeptides and non-human animals expressing humanized MHC class II β chain polypeptides are described in U.S. Patent Nos. 8,847,005 and 9,043,996 and U.S. Pat. Pub. No. 2014/0245467.

[0107] Genetically modified non-human animals comprising exogenous TdT, humanized TCR loci and humanized MHC I and/or MHC II (MHC IIα/IIβ) loci can be generated by breeding using conventional methods; alternatively, they can be generated by homologous recombination in ES cells already comprising one or more genetically engineered loci (*e.g.*, humanized TCR loci), and generating a non-human animal from said ES cells.

[0108] Genetically modified non-human animals comprising exogenous TdT, humanized CAR loci and humanized MHC I and/or MHC II (MHC IIα/IIβ) loci can be generated by breeding using conventional methods; alternatively, they can be generated by homologous recombination in ES cells already comprising one or more genetically engineered loci (*e.g.*, humanized CAR loci), and generating a non-human animal from said ES cells.

[0109] In some aspects, the genetically modified non-human animals and ES cells described herein that express exogenous TdT, humanized TCRs or CARs also express and/or comprise in their genome a locus encoding a humanized CD8 α chain polypeptide. In some aspects, the humanized CD8 α chain polypeptide is fully human. In some aspects, the humanized CD8 α chain polypeptide may comprise a human extracellular immunoglobulin domain and a cytoplasmic domain of endogenous species origin. Humanized CD8 α chain polypeptides, loci encoding humanized CD8 α chain polypeptides and non-human animals expressing humanized CD8 α chain polypeptides are described in U.S. Pat. Pub. Nos. 2014/0245466.

[0110] In some aspects, the genetically modified non-human animals and ES cells described herein that express exogenous TdT, humanized TCRs or CARs may also express and/or comprise in their genome a locus encoding a humanized CD8 β chain polypeptide. In some aspects, the humanized CD8 β chain polypeptide is fully human. In some aspects, the humanized CD8 β chain polypeptide comprises a human extracellular immunoglobulin domain and a cytoplasmic domain of endogenous species origin. Humanized CD8 β chain polypeptides, loci encoding humanized CD8 β chain polypeptides and non-human animals expressing humanized CD8 β chain polypeptides are described in U.S. Pat. Pub. Nos. 2014/0245466.

[0111] In some aspects, the genetically modified non-human animals and ES cells described herein that express exogenous TdT, humanized TCRs or CARs also express and/or comprise in their genome a locus encoding a humanized CD4 polypeptide. In some aspects, the humanized CD4 polypeptide is fully human. In some aspects, the humanized CD4 polypeptide comprises at least one human extracellular immunoglobulin domain and a cytoplasmic domain of endogenous species origin. In some aspects, the humanized CD4 polypeptide comprises at least a human D1 immunoglobulin domain, a human D2 immunoglobulin domain, and a human D3 immunoglobulin domain, and a cytoplasmic domain of endogenous species origin. In some aspects, the humanized CD4 polypeptide comprises a human D1 immunoglobulin domain, a human D2 immunoglobulin domain, a human D3 immunoglobulin domain, a D4 immunoglobulin domain of endogenous species origin and a cytoplasmic domain of endogenous species origin. Humanized CD4 polypeptides, loci encoding humanized CD4 polypeptides and non-human animals expressing humanized CD4 polypeptides are described in U.S. Pat. Pub. Nos. 2014/0245466.

[0112] Genetically modified non-human animals comprising exogenous TdT, humanized TCR loci and humanized CD4 and/or CD8 (CD8α/CD8β) loci can be generated by breeding using conventional methods; alternatively, they can be generated by homologous recombination in ES cells already comprising one or more genetically engineered loci (*e.g.*, humanized TCR loci), and generating a non-human animal from said ES cells.

[0113] Genetically modified non-human animals comprising exogenous TdT, humanized CAR loci and humanized CD4 and/or CD8 (CD8α/CD8β) loci can be generated by breeding using conventional methods; alternatively, they can be generated by homologous recombination in ES cells already comprising one or more genetically engineered loci (*e.g.*, humanized CAR loci), and generating a non-human animal from said ES cells.

Methods of Using the Genetically Modified Rodents

[0114] In certain aspects, provided herein are methods of using the genetically modified rodents described herein to generate antigen binding proteins (*e.g.*, antibodies, CARs, TCRs), cells expressing such antigen binding proteins (*e.g.,* B cells, T cells, B cell hybridomas, T cell hybridomas) and nucleic acids encoding such antigen binding proteins or portions thereof (*e.g.*, variable domains). In some aspects, provided herein are methods of making more diverse antigen binding proteins (*e.g.*, antibodies, CARs, TCRs). In some aspects, provided herein are methods of making rearranged variable regions of antigen binding proteins (*e.g.*, antibodies, CARs, TCRs) that have increased numbers of nucleotide additions.

[0115] In certain aspects, the method comprises exposing a genetically modified non-human animal described herein that has been modified to express exogenous TdT and antibodies or antigen-binding fragments thereof having human variable domains to an antigen such that the genetically modified non-human animal produces an antibody or an antigen-binding fragment thereof comprising a human variable domain specific for the antigen.

[0116] In some aspects the method comprises exposing a genetically modified non-human animal described herein that has been modified to express exogenous TdT and antibodies or antigen-binding fragments thereof having human variable domains to an antigen; and obtaining a B cell expressing an antibody or an antigen-binding fragment thereof comprising a human variable domain specific for the antigen from the non-human animal.

[0117] In some aspects, the method comprises exposing a genetically modified non-human animal described herein that has been modified to express exogenous TdT and antibodies or antigen-binding fragments thereof having human variable domains to an antigen; obtaining a B cell expressing an antibody or an antigen-binding fragment thereof comprising a human variable domain specific for the antigen from the non-human animal; and making a hybridoma from the B cell.

[0118] In some aspects, the method comprises exposing a genetically modified non-human animal described herein that has been modified to express exogenous TdT and antibodies or antigen-binding fragments thereof having human variable domains to an antigen; and obtaining a nucleic acid encoding a human immunoglobulin variable domain specific for the antigen from the non-human animal.

[0119] In certain aspects, the method comprises exposing a genetically modified non-human animal described herein that has been modified to express exogenous TdT and antibodies or antigen-binding fragments thereof having human variable domains to an antigen; obtaining a B cell expressing an antibody or an antigen-binding fragment thereof comprising a human variable domain specific for the antigen from the non-human animal; optionally making a hybridoma from the B cell; and obtaining a nucleic acid encoding a human immunoglobulin variable domain specific for the antigen from the B cell or the hybridoma.

[0120] In some aspects, the method comprises exposing a non-human animal described herein that has been modified to express exogenous TdT and antibodies or antigen-binding fragments thereof having human variable domains to an antigen; obtaining a B cell expressing an antibody or an antigen-binding fragment thereof comprising a human variable domain specific for the antigen from the non-human animal; optionally making a hybridoma from the B cell; obtaining a nucleic acid encoding a human immunoglobulin variable domain specific for the antigen from the B cell or the hybridoma; operably linking the nucleic acid encoding the immunoglobulin variable domain with a nucleic acid encoding a human immunoglobulin constant domain in a host cell; and culturing the host cell under conditions such that the host cell expresses a human antibody comprising the immunoglobulin variable domain and the immunoglobulin constant domain.

[0121] In some aspects, the method comprises exposing a genetically modified non-human animal described herein that has been modified to express exogenous TdT and TCR having human variable domains to an antigen comprising a peptide or a nucleic acid encoding an antigen comprising a peptide such that the peptide is presented on a MHC in the non-human animal; and obtaining a T cell expressing a TCR specific for the peptide presented on the MHC from the genetically modified non-human animal.

[0122] In some aspects, the method comprises exposing a genetically modified non-human animal described herein that has been modified to express exogenous TdT and TCR having human variable domains to an antigen comprising a peptide or a nucleic acid encoding an antigen comprising a peptide such that the peptide is presented on a MHC in the non-human animal; obtaining a T cell expressing a TCR specific for the peptide presented on the MHC from the genetically modified non-human animal; and making a T cell hybridoma from the T cell.

[0123] In some aspects, the method comprises exposing a non-human animal described herein that has been modified to express exogenous TdT and TCR having human variable domains to an antigen comprising a peptide or a nucleic acid encoding an antigen comprising a peptide such that the peptide is presented on a MHC in the non-human animal; obtaining a T cell expressing a TCR specific for the peptide presented on the MHC from the genetically modified non-human animal; and isolating a nucleic acid encoding a human TCR variable domain of the TCR from the T cell.

[0124] In some aspects, the method comprises exposing a non-human animal described herein that has been modified to express exogenous TdT and TCR having human variable domains to an antigen comprising a peptide or a nucleic acid encoding an antigen comprising a peptide such that the peptide is presented on a MHC in the non-human animal; obtaining a T cell expressing a TCR specific for the peptide presented on the MHC from the genetically modified non-human animal; isolating a nucleic acid encoding a TCR variable domain of the TCR from the T cell; and operably linking the nucleic acid encoding the TCR variable domain with a TCR constant domain in a cell such that the cell expresses a TCR comprising the TCR variable domain and the TCR constant domain.

[0125] In some aspects, the method comprise exposing a genetically modified non-human animal described herein that has been modified to express exogenous TdT and CAR having human variable domains to an antigen comprising a peptide or a nucleic acid encoding an antigen comprising a peptide such that the peptide is presented on a MHC in the non-human animal; and obtaining a T cell expressing a CAR specific for the peptide presented on the MHC from the genetically modified non-human animal.

[0126] In some aspects, the method comprises exposing a genetically modified non-human animal described herein that has been modified to express exogenous TdT and CAR having human variable domains to an antigen comprising a peptide or a nucleic acid encoding an antigen comprising a peptide such that the peptide is presented on a MHC in the non-human animal; obtaining a T cell expressing a CAR specific for the peptide presented on the MHC from the genetically modified non-human animal; and making a T cell hybridoma from the T cell.

[0127] In some aspects, the method comprises exposing a non-human animal described herein that has been modified to express exogenous TdT and CAR having human variable domains to an antigen comprising a peptide or a nucleic acid encoding an antigen comprising a peptide such that the peptide is presented on a MHC in the non-human animal; obtaining a T cell expressing a chimeric antigen receptor (CAR) specific for the peptide presented on the MHC from the genetically modified non-human animal; and isolating a nucleic acid encoding a human TCR variable domain of the CAR from the T cell.

[0128] In some aspects, the method comprises exposing a non-human animal described herein that has been modified to express exogenous TdT and CAR having human variable domains to an antigen comprising a peptide or a nucleic acid encoding an antigen comprising a peptide such that the peptide is presented on a MHC in the non-human animal; obtaining a T cell expressing a chimeric antigen receptor (CAR) specific for the peptide presented on the MHC from the genetically modified non-human animal; isolating a nucleic acid encoding a human immunoglobulin variable domain of the CAR from the T cell; and operably linking the nucleic acid encoding the human immunoglobulin variable domain with a human immunoglobulin constant domain in a cell such that the cell expresses an antibody comprising the human immunoglobulin variable domain and the human immunoglobulin constant domain.

[0129] In certain aspects, the methods described herein include a step in which a non-human animal described herein is exposed to an antigen (immunized) in order to induce an immune response (*e.g.,* a B cell immune response and/or a T cell immune response). In some aspects the genetically modified non-human animal is immunized with a whole protein antigen or a fragment thereof. Rodents can be immunized by any method known in the art (see, for example, Harlow and Lane (1988) Antibodies: A Laboratory Manual 1988 Cold Spring Harbor Laboratory; Malik and Lillehoj (1994) Antibody Techniques, Academic Press, CA).

[0130] In some aspects, the genetically modified non-human animal is exposed to the antigen by administering to the non-human animal a virus (*e.g.,* a retrovirus, an adenovirus, a vaccinia virus or a lentivirus) comprising a nucleic acid sequence encoding the antigen. Methods for viral vaccination are provided, for example, in U.S. Pat. Nos. 6,001,349, 8,663,622, 8,691,502, 8,377,688, as well as Precopio et al., JEM204:1405-1416 (2007) In some aspects, the non-human animal is administered the virus directly. In some aspects, a cell (*e.g.,* an antigen presenting cell, such as a dendritic cell) is infected with the virus *in vitro* or *ex* vivo which is then administered to the non-human animal. In some aspects, the virus encodes a peptide/MHC complex (*e.g.,* a single-chain peptide/MHC complex). Examples of single-chain peptide/MHC based vaccines are provided in Truscott et al., J. Immunol. 178:6280-6289 (2007), EP1773383, Kim et al., Vaccine 30:2178-2186 (2012), Kim et al., J. Immunol. 184:4423-4430 (2010)

[0131] In some aspects, the genetically modified non-human animal is exposed to the antigen by administering to the animal a nucleic acid encoding the antigen. In some aspects, the non-human animal is administered a nucleic acid encoding a single chain peptide/MHC complex. Examples of single-chain peptide/MHC based vaccines are provided in Truscott et al., J.

Immunol. 178:6280-6289 (2007), EP1773383, Kim et al., Vaccine 30:2178-2186 (2012), Kim et al., J. Immunol. 184:4423-4430 (2010) In certain aspects, the nucleic acid is a DNA vector. The delivery of nucleic acids can be by any technique known in the art including viral mediated gene transfer and liposome mediated gene transfer. A polynucleotide of interest is associated with a liposome to form a gene delivery vehicle as described in, for example, U.S. Pat. Nos. 6,770,291, 7,001,614, 6,749,863, 5,512,295 and 7,112,338, each of which is hereby incorporated by reference. In some aspects, the nucleic acid is an mRNA vector. Exemplary methods for generating and administering mRNA vectors are described in, for example, U.S. Pat. No. 8,278,036 and U.S. Pat. Pub. Nos. 2013/151736 and 2012/135805

[0132] In some aspects, antigen is a cancer-associated antigen. Examples of cancer-associated antigens include, but are not limited to, adipophilin, AIM-2, ALDH1A1, alpha-actinin-4, alpha-fetoprotein ("AFP"), ALK, ANKRD30A, ARTC1, B-RAF, BAGE-1, BCLX (L), BCR-ABL fusion protein b3a2, beta-catenin, BING-4, BIRC7, CA-125, CA9, CALCA, carcinoembryonic antigen ("CEA"), CALR, CASP-5, CASP-8, CCR5, CD19, CD20, CD22, CD27, CD274, CD30, CD33, CD38, CD40, CD44, CD45, CD52, CD56, CD79, Cdc27, CDK12, CDK4, CDKN2A, CEA, CLEC12A, CLPP, COA-1, CPSF, CSNK1A1, CTAG1, CTAG2, cyclin D1, Cyclin-A1, dek-can fusion protein, DKK1, EFTUD2, EGFR, EGFR variant III, Elongation factor 2, ENAH (hMena), Ep-CAM, EpCAM, EphA2, EphA3, epithelial tumor antigen ("ETA"), ERBB3, ERBB4, ETV6-AML1 fusion protein, EZH2, FCRL3, FGF5, FLT3-ITD, FN1, FOLR1, G250/MN/CAIX, GAGE-1,2,8, GAGE-3,4,5,6,7, GAS7, glypican-3, GnTV, gp100/Pmel17, GPNMB, GM3, GPR112, IL3RA, HAUS3, Hepsin, HER-2/neu, HERV-K-MEL, HLA-A11, HLA-A2, HLA-DOB, hsp70-2, IDO1, IGF2B3, IL13Ralpha2, Intestinal carboxyl esterase, K-ras, Kallikrein 4, KIF20A, KIT, KK-LC-1, KKLC1, KM-HN-1, KMHN1 also known as CCDC110, KRAS, LAGE-1, LDLR-fucosyltransferaseAS fusion protein, Lengsin, LGR5, LMP2, M-CSF, MAGE-A1, MAGE-A10, MAGE-A12, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-C1, MAGE-C2, malic enzyme, mammaglobin-A, MART2, MATN, MC1R, MCSP, mdm-2, ME1, Melan-A/MART-1, Meloe, Midkine, MMP-2, MMP-7, MUC1, MUC2, MUC3, MUC4, MUC5, MUC5AC, MUC16, mucin, MUM-1, MUM-2, MUM-3, Myosin, Myosin class I, N-raw, NA88-A, neo-PAP, NFYC, NY-BR-1, NY-ESO-1/LAGE-2, OA1, OGT, OS-9, OX40, P polypeptide, p53, PAP, PAX3, PAX5, PBF, PLAC1, PMEL, pml-RARalpha fusion protein, polymorphic epithelial mucin ("PEM"), PPP1R3B, PRAME, PRDX5, PRLR, PSA, PSMA, PTPRK, RAB38/NY-MEL-1, RAGE-1, RBAF600, RET, RGS5, RhoC, RNF43, ROR1, RU2AS, SAGE, SART1, SART3, secernin 1, SIRT2, SLAMF7, SLC39A6, SNRPD1, SOX10, Sp17, SPA17, SSX-2, SSX-4, STEAP1, STEAP2, survivin, SYT-SSX1 or -SSX2 fusion protein, TAG-1, TAG-2, Telomerase, TERT, TGF-betaRII, Thompson-nouvelle antigen, TMPRSS2, TNFRSF17, TPBG, TRAG-3, Triosephosphate isomerase, TRP-1/gp75, TRP-2, TRP2-INT2, tyrosinase, tyrosinase ("TYR"), UPK3A, VEGF, VTCN1, WT1, XAGE-1b/GAGED2a. In some aspects, the antigen is a neo-antigen.

[0133] In some aspects, the antigen is an antigen expressed by an infectious pathogen. In some aspects, the pathogen is a virus, a bacteria, a fungus, a helminth, or a protozoa. Nonlimiting examples of viruses include HIV, hepatitis A, hepatitis B, hepatitis C, herpes virus (*e.g.*, HSV-1, HSV-2, CMV, HAV-6, VZV, Epstein Barr virus), adenovirus, influenza virus, flavivirus, echovirus, rhinovirus, coxsackie virus, coronavirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus, ebola virus, and arboviral encephalitis virus antigen.. In some aspects, the parasite is malaria. In some aspects, pathogen is *Aspergillus, Brugia, Candida, Chlamydia, Coccidia, Cryptococcus, Dirofilaria, Gonococcus, Histoplasma, Klebsiella, Legionella, Leishmania, Meningococci, Mycobacterium, Mycoplasma, Paramecium, Pertussis, Plasmodium, Pneumococcus, Pneumocystis, Pseudomonas, Rickettsia, Salmonella, Shigella, Staphylococcus, Streptococcus, Toxoplasma* and *Vibriocholerae.* Exemplary species include *Neisseria gonorrhea, Mycobacterium tuberculosis, Candida albicans, Candida tropicalis, Trichomonas vaginalis, Haemophilus vaginalis,* Group B *Streptococcus sp., Microplasma hominis, Hemophilus ducreyi, Granuloma inguinale, Lymphopathia venereum, Treponema pallidum, Brucella abortus. Brucella melitensis, Brucella suis, Brucella canis, Campylobacter fetus, Campylobacter fetus intestinalis, Leptospira pomona, Listeria monocytogenes, Brucella ovis, Chlamydia psittaci, Trichomonas foetus, Toxoplasma gondii, Escherichia coli, Actinobacillus equuli, Salmonella abortus ovis, Salmonella abortus equi, Pseudomonas aeruginosa, Corynebacterium equi, Corynebacterium pyogenes, Actinobaccilus seminis, Mycoplasma bovigenitalium, Aspergillus fumigatus, Absidia ramosa, Trypanosoma equiperdum, Babesia caballi, Clostridium tetani, Clostridium botulinum;* or, a fungus, such as, *e.g., Paracoccidioides brasiliensis;* or other pathogen, *e.g., Plasmodium falciparum.*

[0134] In some aspects of the methods described herein, the method includes the step of obtaining a T cell and/or or B cell from the genetically modified non-human animal. In certain aspects, any method known in the art can be used to obtain such cells. For example, such T cells and/or B cells can be obtained from the spleen, lymph nodes and/or peripheral blood of the animal. Such T cells and/or B cells can be screened for binding specificity using methods available in the art.

[0135] In some aspects, the methods described herein include the step of making a B cell hybridoma from a B cell. Methods useful for making a B cell hybridoma are known in the art and described, for example, in Harlow and Lane (1988) Antibodies: A Laboratory Manual 1988 Cold Spring Harbor Laboratory; Malik and Lillehoj (1994) Antibody Techniques, Academic Press, CA.

[0136] In some aspects, the methods described herein include the step of making a T cell hybridoma from a T cell. Methods useful for making a T cell hybridoma are known in the art and described, for example, in Hedrick et al., Cell 30:141-152 (1982) and Kruisbeek Curr. Protoc. Immunol. Chapter 3 (2001) and White et al., Methods in Molecular Biology 134:185-193 (2000).

[0137] In some aspects, the methods provided herein include the step of isolating a nucleic acid encoding an Ig or TCR variable region. In some aspects of the methods described herein, any method can be used to isolate the nucleic acid encoding the Ig or TCR variable region.

[0138] In some aspects, the step of isolating the nucleic acid comprises making a B cell or T cell hybridoma from a B cell or T cell respectively and isolating the nucleic acid from the hybridoma. In some aspects, the nucleic acid is isolated using a nucleic acid amplification process. For example, in some aspects the nucleic acid amplification process is polymerase chain reaction (PCR), ligase chain reaction (LCR), strand displacement amplification (SDA), transcription mediated amplification (TMA), self-sustained sequence replication (3SR), Qβ replicase based amplification, nucleic acid sequence-based amplification (NASBA), repair chain reaction (RCR), boomerang DNA amplification (BDA) or rolling circle amplification (RCA).

[0139] In some aspects, the nucleic acid is isolated by sequencing the rearranged Ig or TCR variable region gene in a B cell, T cell, B cell hybridoma or T cell hybridoma and synthesizing a nucleic acid sequence comprising the rearranged Ig or TCR variable region gene. Exemplary nucleic acid sequencing processes include, but are not limited to chain termination sequencing, sequencing by ligation, sequencing by synthesis, pyrosequencing, ion semiconductor sequencing, single-molecule real-time sequencing, 454 sequencing, and/or Dilute-'N'-Go sequencing.

[0140] When DNA fragments encoding heavy and/or light chain Ig variable regions are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a variable region-encoding DNA fragment is operably linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operably linked", as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

[0141] The isolated DNA encoding the heavy chain variable region can be converted to a full-length heavy chain gene by operably linking the variable region-encoding DNA to another DNA molecule encoding heavy chain constant domain (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see *e.g.,* Kabat, E. A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, or Lefranc, The Immunoglobulin Handbook, London: Academic Press 2001) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant domain can be, for example, an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant domain. For a Fab fragment heavy chain gene, the V_H-encoding DNA can be operably linked to another DNA molecule encoding only the heavy chain CH1 constant region.

[0142] Thus, in some aspects, the methods described herein include the step of operably linking a nucleic acid sequence encoding a heavy chain Ig variable domain with a nucleic acid sequence encoding a heavy chain Ig constant domain in a host cell such that the host cell expresses an Ig heavy chain polypeptide comprising the Ig heavy chain variable domain and the Ig heavy chain constant domain. In some aspects, the method includes the step of operably linking a nucleic acid sequence encoding a light chain Ig variable domain with a nucleic acid sequence encoding a light chain Ig constant domain in a host cell such that the host cell expresses an Ig light chain polypeptide comprising the Ig light chain variable domain and the Ig heavy chain constant domain. In some aspects, the method includes the step of operably linking a nucleic acid sequence encoding a heavy chain Ig variable domain with a nucleic acid sequence encoding a heavy chain Ig constant domain in a host cell and operably linking a nucleic acid sequence encoding a light chain Ig variable domain with a nucleic acid sequence encoding a light chain Ig constant domain in the host cell such that the host cell expresses an antibody having a heavy chain comprising the heavy chain Ig variable domain and the heavy chain Ig constant domain and a light chain comprising the light chain Ig variable domain and the light chain Ig constant domain. Ig variable regions can be linked with Ig constant regions using standard molecular biology techniques well known in the art. In some aspects, any host cell capable of expressing an immunoglobulin polypeptide can be used. In some aspects the cell is a CHO cell, a HEK-293 cell, a BHK cell, a NS0 cell, a SP2/0 cell or a Vero cell or a retinal cell expressing a viral nucleic acid sequence (e.g., PERC.6$^{\text{TM}}$ cell).

[0143] In some aspects, the nucleic acid encoding the heavy chain constant domain encodes a constant domain that comprises a modified Fc domain (*e.g.,* a mutation that alters the interaction between the Fc and a Fc receptor). For example, in some aspects, the constant domain comprises modification to its Fc domain at position 235, 236, 237, 239, 265, 267, 268, 269, 270, 298, 326, 327, 330, 332, 350, 351, 366, 392, 394, 405 and/or 407 (using the EU numbering system). In some aspects, the modification is selected from the group consisting of L235A, G236E, G237F, S239E, S239D, D265E, D265S, S267E, S267D, S267G, H268E, H268D, E269L, D270N, D270E, S298A, K326A, K326D, A327H, A327V, A327L, A330I, A330S, I332E, T350V, L351Y, T366L, K392M, K392L, T394W, F405A and/or Y407V (using the EU numbering system). In some aspects, the constant domain comprises multiple modifications to its Fc domain. In some aspects, the multiple modifications are selected from the group consisting of D270N/K326D, S239E/S298A/K326A/A327H, L235A/S239E/D265E/A327H, G236E/G237F/S239E, G237F/S239E/D265E, G327F/S239E/H268D, G236E/D270N/A327V/I332E, G237F/S239E/A327H, G237F/A327L/A330I, S239D/D265S/S298A/I332E, S239E/D265S/H268D/I332E, S239E/D265S/I332E, S239E/S267E/H268D, S239E/A327L/A330I, D265E/S267D/A330S, S267G/H268E/D270E, H268D/E269L/S298A/K326A/A327H, H268D//K326A/A327H. Additional Fc modifications and combinations of Fc modifications are provided in U.S. Patent Nos. 5,624,821, 5,648,260, 6,528,624, 6,737,056, 7,122,637, 7,183,387, 7,297,775, 7,317,091, 7,332,581, 7,632,497, 7,662,925, 7,695,936, 8,093,359, 8,216,805, 8,218,805, 8,388,955 and 8,937,158, and U.S. Patent Publication Nos. 2005/0054832, 2006/0222653, 2006/0275282, 2006/0275283, 2007/0190063, 2008/0154025, 2009/0042291 2013/0108623 and 2013/0089541.

Antigen Binding Proteins

[0144] In certain aspects, provided herein are antigen binding proteins (*e.g.*, antibodies, TCRs, CARs and antigen binding fragments thereof) obtainable and/or obtained according to a method described herein (*e.g.*, using a non-human animal described herein).

[0145] In certain aspects, the antigen binding molecules provided herein are able to specifically bind a target antigen with a dissociation constant of no greater than 10^{-6}, 10^{-7}, 10^{-8} or 10^{-9} M. In some aspects, the binding affinity of the antigen binding protein to an antigen (as expressed by K_D) is at least 10 fold less, at least 100 fold less or at least 1000 fold less than the affinity of the antigen binding protein for an unrelated antigen. In some aspects, the antigen binding protein binds to a peptide/MHC complex with a dissociation constant of no greater than 10^{-6}, 10^{-7}, 10^{-8} or 10^{-9} M. In some aspects, the binding affinity of the antigen binding protein to a peptide/MHC complex (as expressed by K_D) is at least 10 fold less, at least 100 fold less or at least 1000 fold less than the affinity of the antigen binding protein for the peptide for the same MHC protein presenting an unrelated peptide. Standard assays to evaluate the binding ability of the antigen binding proteins are known in the art, including for example, ELISAs, Western blots and RIAs. The binding kinetics (*e.g.*, binding affinity) of the antigen binding protein also can be assessed by standard assays known in the art, such as by Biacore analysis.

[0146] In some aspects, the antigen comprises an epitope of and/or is a cancer-associated antigen. Examples of cancer-associated antigens include, but are not limited to, adipophilin, AIM-2, ALDH1A1, alpha-actinin-4, alpha-fetoprotein ("AFP"), ARTC1, B-RAF, BAGE-1, BCLX (L), BCR-ABL fusion protein b3a2, beta-catenin, BING-4, CA-125, CALCA, carcinoembryonic antigen ("CEA"), CASP-5, CASP-8, CD274, CD45, Cdc27, CDK12, CDK4, CDKN2A, CEA, CLPP, COA-1, CPSF, CSNK1A1, CTAG1, CTAG2, cyclin D1, Cyclin-A1, dek-can fusion protein, DKK1, EFTUD2, Elongation factor 2, ENAH (hMena), Ep-CAM, EpCAM, EphA3, epithelial tumor antigen ("ETA"), ETV6-AML1 fusion protein, EZH2, FGF5, FLT3-ITD, FN1, G250/MN/CAIX, GAGE-1,2,8, GAGE-3,4,5,6,7, GAS7, glypican-3, GnTV, gp100/Pmel17, GPNMB, HAUS3, Hepsin, HER-2/neu, HERV-K-MEL, HLA-A11, HLA-A2, HLA-DOB, hsp70-2, IDO1, IGF2B3, IL13Ralpha2, Intestinal carboxyl esterase, K-ras, Kallikrein 4, KIF20A, KK-LC-1, KKLC1, KM-HN-1, KMHN1 also known as CCDC110, LAGE-1, LDLR-fucosyltransferaseAS fusion protein, Lengsin, M-CSF, MAGE-A1, MAGE-A10, MAGE-A12, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-C1, MAGE-C2, malic enzyme, mammaglobin-A, MART2, MATN, MC1R, MCSP, mdm-2, ME1, Melan-A/MART-1, Meloe, Midkine, MMP-2, MMP-7, MUC1, MUC5AC, mucin, MUM-1, MUM-2, MUM-3, Myosin, Myosin class I, N-raw, NA88-A, neo-PAP, NFYC, NY-BR-1, NY-ESO-1/LAGE-2, OA1, OGT, OS-9, P polypeptide, p53, PAP, PAX5, PBF, pml-RARalpha fusion protein, polymorphic epithelial mucin ("PEM"), PPP1R3B, PRAME, PRDX5, PSA, PSMA, PTPRK, RAB38/NY-MEL-1, RAGE-1, RBAF600, RGS5, RhoC, RNF43, RU2AS, SAGE, secernin 1, SIRT2, SNRPD1, SOX10, Sp17, SPA17, SSX-2, SSX-4, STEAP1, survivin, SYT-SSX1 or -SSX2 fusion protein, TAG-1, TAG-2, Telomerase, TGF-betaRII, TPBG, TRAG-3, Triosephosphate isomerase, TRP-1/gp75, TRP-2, TRP2-INT2, tyrosinase, tyrosinase ("TYR"), VEGF, WT1, XAGE-1b/GAGED2a. In some aspects, the antigen is a neo-antigen.

[0147] In some aspects, the antigen comprises an epitope of and/or is an antigen expressed by an infectious pathogen. In some aspects, the pathogen is a virus, a bacteria, a fungus, a helminth, or a protozoa. Some nonlimiting examples of viruses include HPV, HBV, hepatitis C

Virus (HCV), retroviruses such as human immunodeficiency virus (HIV-1 and HIV-2), herpes viruses such as Epstein Barr Virus (EBV), cytomegalovirus (CMV), HSV-1 and HSV-2, and influenza virus. In some aspects, the parasite is malaria. In some aspects, pathogen is *Aspergillus, Brugia, Candida, Chlamydia, Coccidia, Cryptococcus, Dirofilaria, Gonococcus, Histoplasma, Leishmania, Mycobacterium, Mycoplasma, Paramecium, Pertussis, Plasmodium, Pneumococcus, Pneumocystis, Rickettsia, Salmonella, Shigella, Staphylococcus, Streptococcus, Toxoplasma* and *Vibriocholerae.* Exemplary species include *Neisseria gonorrhea, Mycobacterium tuberculosis, Candida albicans, Candida tropicalis, Trichomonas vaginalis, Haemophilus vaginalis,* Group B *Streptococcus sp., Microplasma hominis, Hemophilus ducreyi, Granuloma inguinale, Lymphopathia venereum, Treponema pallidum, Brucella abortus. Brucella melitensis, Brucella suis, Brucella canis, Campylobacter fetus, Campylobacter fetus intestinalis, Leptospira pomona, Listeria monocytogenes, Brucella ovis, Chlamydia psittaci, Trichomonas foetus, Toxoplasma gondii, Escherichia coli, Actinobacillus equuli, Salmonella abortus ovis, Salmonella abortus equi, Pseudomonas aeruginosa, Corynebacterium equi, Corynebacterium pyogenes, Actinobaccilus seminis, Mycoplasma bovigenitalium, Aspergillus fumigatus, Absidia ramosa, Trypanosoma equiperdum, Babesia caballi, Clostridium tetani, Clostridium botulinum;* or, a fungus, such as, *e.g., Paracoccidioides brasiliensis;* or other pathogen, *e.g., Plasmodium falciparum.*

[0148] In some aspects, the antigen comprises an epitope of and/or is a protein that is the target of an autoreactive T cell in an inflammatory disease, skin or organ transplantation rejection, graft-versus-host disease (GVHD), or autoimmune diseases. Examples of autoimmune diseases include, for example, glomerular nephritis, arthritis, dilated cardiomyopathy-like disease, ulceous colitis, Sjogren syndrome, Crohn disease, systemic erythematodes, chronic rheumatoid arthritis, multiple sclerosis, psoriasis, allergic contact dermatitis, polymyosiis, pachyderma, periarteritis nodosa, rheumatic fever, vitiligo vulgaris, insulin dependent diabetes mellitus, Behcet disease, Hashimoto disease, Addison disease, dermatomyositis, myasthenia gravis, Reiter syndrome, Graves' disease, anaemia perniciosa, Goodpasture syndrome, sterility disease, chronic active hepatitis, pemphigus, autoimmune thrombopenic purpura, and autoimmune hemolytic anemia, active chronic hepatitis, Addison's disease, anti-phospholipid syndrome, atopic allergy, autoimmune atrophic gastritis, achlorhydra autoimmune, celiac disease, Cushing's syndrome, dermatomyositis, discoid lupus, erythematosis, Goodpasture's syndrome, Hashimoto's thyroiditis, idiopathic adrenal atrophy, idiopathic thrombocytopenia, insulin-dependent diabetes, Lambert-Eaton syndrome, lupoid hepatitis, some cases of lymphopenia, mixed connective tissue disease, pemphigoid, pemphigus vulgaris, pernicious anema, phacogenic uveitis, polyarteritis nodosa, polyglandular autosyndromes, primary biliary cirrhosis, primary sclerosing cholangitis, Raynaud's syndrome, relapsing polychondritis, Schmidt's syndrome, limited scleroderma (or crest syndrome), sympathetic ophthalmia, systemic lupus erythematosis, Takayasu's arteritis, temporal arteritis, thyrotoxicosis, type b insulin resistance, ulcerative colitis and Wegener's granulomatosis. Exemplary proteins include targeted by autoreactive T cells include, for example, p205, insulin, thyroid-stimulating hormone, tyrosinase, TRP1, and myelin.

[0149] In some aspects, the antigen binding protein is an antibody. In some aspects, the antibodies provided herein comprise human heavy chain variable domains. In some aspects, the antibodies comprise human heavy chain constant domains. In some aspects, the antibodies provided herein comprise a IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant domain. The sequences of human heavy chain constant domains are known in the art (see *e.g.,* Kabat, E. A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, or Lefranc, The Immunoglobulin Handbook, London: Academic Press 2001). In some aspects, the antibodies provided herein lack a heavy chain constant domain or a portion thereof.

[0150] In some aspects, the antibodies provided herein comprise a modified Fc domain (*e.g.,* a mutation that alters the interaction between the Fc and a Fc receptor). For example, in some aspects, the antibodies provided herein comprise modification to their Fc domain at position 235, 236, 237, 239, 265, 267, 268, 269, 270, 298, 326, 327, 330, 332, 350, 351, 366, 392, 394, 405 and/or 407 (using the EU numbering system). In some aspects, the modification is selected from the group consisting of L235A, G236E, G237F, S239E, S239D, D265E, D265S, S267E, S267D, S267G, H268E, H268D, E269L, D270N, D270E, S298A, K326A, K326D, A327H, A327V, A327L, A330I, A330S, I332E, T350V, L351Y, T366L, K392M, K392L, T394W, F405A and/or Y407V (using the EU numbering system). In some aspects, the antibodies comprise multiple modifications to their Fc domain. In some aspects, the multiple modifications are selected from the group consisting of D270N/K326D, S239E/S298A/K326A/A327H, L235A/S239E/D265E/A327H, G236E/G237F/S239E, G237F/S239E/D265E, G327F/S239E/H268D, G236E/D270N/A327V/I332E, G237F/S239E/A327H, G237F/A327L/A330I, S239D/D265S/S298A/I332E, S239E/D265S/H268D/I332E, S239E/D265S/I332E, S239E/S267E/H268D, S239E/A327L/A330I, D265E/S267D/A330S, S267G/H268E/D270E, H268D/E269L/S298A/K326A/A327H, H268D//K326A/A327H. Additional Fc modifications and combinations of Fc modifications are provided in U.S. Patent Nos. 5,624,821, 5,648,260, 6,528,624, 6,737,056, 7,122,637, 7,183,387, 7,297,775, 7,317,091, 7,332,581, 7,632,497, 7,662,925, 7,695,936, 8,093,359, 8,216,805, 8,218,805, 8,388,955 and 8,937,158, and U.S. Patent Publication Nos. 2005/0054832, 2006/0222653, 2006/0275282, 2006/0275283, 2007/0190063, 2008/0154025, 2009/0042291 2013/0108623 and 2013/0089541.

[0151] In some aspects, the antibody is a bi-specific antibody. In some aspects, the two antigen binding domains of the bi-specific antibody have distinct heavy chain variable domains but have identical light chain variable domains. In some aspects, the Fc domains of the heavy chains comprise modifications to facilitate heavy chain heterodimer formation and/or to inhibit heavy chain homodimer formation. Such modifications are provided, for example, in U.S. Pat. Nos. 5,731,168, 5,807,706, 5,821,333, 7,642,228 and 8,679,785 and in U.S. Pat. Pub. No. 2013/0195849.

[0152] In some aspects, the antibodies provided herein have human light chain variable domains. In some embodiments, the light chain variable domains are λ light chain variable domains. In some embodiments, the light chain variable domains are κ light chain variable domains. In some embodiments, the antibodies have human light chain constant domains. In some embodiments, the light chain constant domains are λ light chain constant domains. In some embodiments, the light chain constant domains are κ light chain constant domains. The sequences of human light chain constant domains are known in the art (see *e.g.,* Kabat, E. A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, or Lefranc, The Immunoglobulin Handbook, London: Academic Press 2001).

[0153] In some embodiments, the antibodies described herein are intact antibodies. In some embodiments, the antibodies described herein are antibody fragments that retain antigen binding. In some embodiments, the antibody fragment is a Fab, Fab', $F(ab')_2$, Fv, scFv, disulfide linked Fv, Fd, single-chain antibodies, isolated CDRH3 or another antibody fragment that retain at least a portion of the variable domain of an intact antibody.

[0154] In certain aspects, the antigen binding protein is a CAR. In some aspects, the CAR is membrane-bound. In some aspects, the CAR is a soluble CAR (*e.g.,* lacking a transmembrane or cytoplasmic domain). In some aspects, such CARs comprise a first CAR polypeptide comprising an Ig heavy chain variable domain and a TCRβ constant domain and a second CAR polypeptide comprising an Ig light chain variable domain (*e.g.*, an Ig κ variable domain or an Ig λ variable domain) and a TCRα constant domain. In some aspects, the Ig heavy chain variable domain and/or the Ig light chain variable domain are human Ig variable domains. In some aspects, the TCRβ constant domain and/or the TCRα constant domain are non-human constant domains (*e.g.*, rat or mouse constant domains). In some aspects, the TCRβ constant domain and/or the TCRα constant domain are human constant domains.

[0155] In certain aspects, the antigen binding protein is a TCR. In some embodiments, the TCR is membrane-bound. In some aspects, the TCR is a soluble TCR (*e.g.,* lacking a transmembrane or cytoplasmic domain). In some aspects, such TCRs comprise a first TCR polypeptide comprising a TCRβ variable domain and a TCRβ constant domain and a second TCR polypeptide comprising a TCRα variable domain and a TCRα constant domain. In some aspects, the TCRα variable domain and/or the TCRβ variable domain are human TCR variable domains. In some aspects, the TCRβ constant domain and/or the TCRα constant domain are non-human constant domains (*e.g.*, rat or mouse constant domains). In some aspects, the TCRβ constant domain and/or the TCRα constant domain are human constant domains.

Pharmaceutical Compositions

[0156] In certain aspects, provided herein is a composition, *e.g.*, a pharmaceutical composition, containing at least one agent described herein (*e.g.,* an antigen binding molecule described herein, such as an antibody, a CAR or a TCR described herein, obtained from the non-human animal described herein) formulated together with a pharmaceutically acceptable carrier.

[0157] The pharmaceutical compositions provided herein may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, *e.g.*, those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; or (2) parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation.

[0158] Pharmaceutical compositions provided herein suitable for parenteral administration comprise one or more agents described herein in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain sugars, alcohols, antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

[0159] Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions provided herein include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0160] In certain aspects, the compositions comprise an antibody, a TCR and/or a CAR described herein in a concentration resulting in a w/v appropriate for a desired dose. The antibody, TCR and/or CAR may be present in the composition at a concentration of at least 1 mg/mL, at least 5 mg/mL, at least 10 mg/mL, at least 15 mg/mL, at least 20 mg/mL, at least 25 mg/mL, at least 30 mg/mL, at least 35 mg/mL, at least 40 mg/mL, at least 45 mg/mL, at least 50 mg/mL, at least 55 mg/mL, at least 60 mg/mL, at least 65 mg/mL, at least 70 mg/mL, at least 75 mg/mL, at least 80 mg/mL, at least 85 mg/mL, at least 90 mg/mL, at least 95 mg/mL, at least 100 mg/mL, at least 105 mg/mL, at least 110 mg/mL, at least 115 mg/mL, at least 120 mg/mL, at least 125 mg/mL, at least 130 mg/mL, at least 135 mg/mL, at least 140 mg/mL, at least 150 mg/mL, at least 200 mg/mL, at least 250 mg/mL, or at least 300 mg/mL.

[0161] In some aspects, the composition comprises one or more active compounds as necessary for the particular indication being treated, typically those with complementary activities that do not adversely affect each other. Such additional active compounds are suitably present in combination in amounts that are effective for the purpose intended.

[0162] In some aspects, compositions are prepared by mixing an antibody, a TCR and/or a CAR described herein with optional physiologically acceptable carriers, excipients or stabilizers, including, but not limited to buffering agents, saccharides, salts, surfactants, solubilizers, polyols, diluents, binders, stabilizers, salts, lipophilic solvents, amino acids, chelators, preservatives, or the like (Goodman and Gilman's The Pharmacological Basis of Therapeutics, 12th edition, L. Brunton, et al. and Remington's Pharmaceutical Sciences, 16th edition, Osol, A.

Ed. (1999)), in the form of lyophilized compositions or aqueous solutions at a desired final concentration. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as histidine, phosphate, citrate, glycine, acetate and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including trehalose, glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEEN, polysorbate 80, PLURONICS® or polyethylene glycol (PEG).

[0163] In some aspects, the buffering agent is histidine, citrate, phosphate, glycine, or acetate. The saccharide excipient may be trehalose, sucrose, mannitol, maltose or raffinose. The surfactant may be polysorbate 20, polysorbate 40, polysorbate 80, or Pluronic F68. The salt may be NaCl, KCl, MgCl2, or CaCl2.

[0164] In some aspects, the composition comprises a buffering or pH adjusting agent to provide improved pH control. Such a composition may have a pH of between about 3.0 and about 9.0, between about 4.0 and about 8.0, between about 5.0 and about 8.0, between about 5.0 and about 7.0, between about 5.0 and about 6.5, between about 5.5 and about 8.0, between about 5.5 and about 7.0, or between about 5.5 and about 6.5. In a further aspect, such a composition has a pH of about 3.0, about 3.5, about 4.0, about 4.5, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7.0, about 7.5, about 8.0, about 8.5, or about 9.0. In a specific aspect, a composition has a pH of about 6.0. One of skill in the art understands that the pH of a composition generally should not be equal to the isoelectric point of the particular antibody, TCR or CAR to be used in the composition. Typically, the buffering agent is a salt prepared from an organic or inorganic acid or base. Representative buffering agents include, but are not limited to, organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. In addition, amino acid components can also function in a buffering capacity. Representative amino acid components which may be utilized in the composition as buffering agents include, but are not limited to, glycine and histidine. In certain aspects, the buffering agent is chosen from histidine, citrate, phosphate, glycine, and acetate. In a specific aspect, the buffering agent is histidine. In another specific aspect, the buffering agent is citrate. In yet another specific aspect, the buffering agent is glycine. The purity of the buffering agent should be at least 98%, or at least 99%, or at least 99.5%. As used herein, the term "purity" in the context of histidine and glycine refers to chemical purity of histidine or glycine as understood in the art, *e.g.,* as described in The Merck Index, 13th ed., O'Neil et al. ed. (Merck & Co., 2001).

[0165] In certain aspects, the composition comprise histidine as a buffering agent. In certain aspects the histidine is present in the composition at a concentration of at least about 1 mM, at least about 5 mM, at least about 10 mM, at least about 20 mM, at least about 30 mM, at least about 40 mM, at least about 50 mM, at least about 75 mM, at least about 100 mM, at least about 150 mM, or at least about 200 mM histidine. In another aspect, a composition comprises between about 1 mM and about 200 mM, between about 1 mM and about 150 mM, between about 1 mM and about 100 mM, between about 1 mM and about 75 mM, between about 10 mM and about 200 mM, between about 10 mM and about 150 mM, between about 10 mM and about 100 mM, between about 10 mM and about 75 mM, between about 10 mM and about 50 mM, between about 10 mM and about 40 mM, between about 10 mM and about 30 mM, between about 20 mM and about 75 mM, between about 20 mM and about 50 mM, between about 20 mM and about 40 mM, or between about 20 mM and about 30 mM histidine. In a further aspect, the composition comprises about 1 mM, about 5 mM, about 10 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM, about 100 mM, about 150 mM, or about 200 mM histidine. In a specific aspect, a composition may comprise about 10 mM, about 25 mM, or no histidine.

[0166] In some aspects, the composition comprises a carbohydrate excipient. Carbohydrate excipients can act, *e.g.,* as viscosity enhancing agents, stabilizers, bulking agents, solubilizing agents, and/or the like. Carbohydrate excipients are generally present at between about 1% to about 99% by weight or volume, *e.g.*, between about 0.1% to about 20%, between about 0.1% to about 15%, between about 0.1% to about 5%, between about 1% to about 20%, between about 5% to about 15%, between about 8% to about 10%, between about 10% and about 15%, between about 15% and about 20%, between 0.1% to 20%, between 5% to 15%, between 8% to 10%, between 10% and 15%, between 15% and 20%, between about 0.1% to about 5%, between about 5% to about 10%, or between about 15% to about 20%. In still other specific aspects, the carbohydrate excipient is present at 1%, or at 1.5%, or at 2%, or at 2.5%, or at 3%, or at 4%, or at 5%, or at 10%, or at 15%, or at 20%.

[0167] In some aspects, the composition comprises a carbohydrate excipient. Carbohydrate excipients suitable for use in the compositions include, but are not limited to, monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol) and the like. In certain aspects, the carbohydrate excipients for use in the compositions provided herein are chosen from sucrose, trehalose, lactose, mannitol, and raffinose. In a specific aspect, the carbohydrate excipient is trehalose. In another specific aspect, the carbohydrate excipient is mannitol. In yet another specific aspect, the carbohydrate excipient is sucrose. In still another specific aspect, the carbohydrate excipient is raffinose. The purity of the carbohydrate excipient should be at least

98%, or at least 99%, or at least 99.5%.

[0168] In some aspects, the composition comprise trehalose. In certain aspects, a composition comprises at least about 1%, at least about 2%, at least about 4%, at least about 8%, at least about 20%, at least about 30%, or at least about 40% trehalose. In another aspect, a composition comprises between about 1% and about 40%, between about 1% and about 30%, between about 1% and about 20%, between about 2% and about 40%, between about 2% and about 30%, between about 2% and about 20%, between about 4% and about 40%, between about 4% and about 30%, or between about 4% and about 20% trehalose. In a further aspect, a composition comprises about 1%, about 2%, about 4%, about 6%, about 8%, about 15%, about 20%, about 30%, or about 40% trehalose. In a specific aspect, a composition comprises about 4%, about 6% or about 15% trehalose.

[0169] In certain aspects, the composition comprise an excipient. In a specific aspect, a composition comprises at least one excipient chosen from: sugar, salt, surfactant, amino acid, polyol, chelating agent, emulsifier and preservative. In certain aspects, a composition comprises a salt, *e.g.,* a salt selected from: NaCl, KCl, CaCl2, and MgCl2. In a specific embodiment, the composition comprises NaCl.

[0170] In some aspects, the composition comprises an amino acid, *e.g.*, lysine, arginine, glycine, histidine or an amino acid salt. The composition may comprise at least about 1 mM, at least about 10 mM, at least about 25 mM, at least about 50 mM, at least about 100 mM, at least about 150 mM, at least about 200 mM, at least about 250 mM, at least about 300 mM, at least about 350 mM, or at least about 400 mM of an amino acid. In another aspect, the composition may comprise between about 1 mM and about 100 mM, between about 10 mM and about 150 mM, between about 25 mM and about 250 mM, between about 25 mM and about 300 mM, between about 25 mM and about 350 mM, between about 25 mM and about 400 mM, between about 50 mM and about 250 mM, between about 50 mM and about 300 mM, between about 50 mM and about 350 mM, between about 50 mM and about 400 mM, between about 100 mM and about 250 mM, between about 100 mM and about 300 mM, between about 100 mM and about 400 mM, between about 150 mM and about 250 mM, between about 150 mM and about 300 mM, or between about 150 mM and about 400 mM of an amino acid. In a further aspect, a composition comprises about 1 mM, 1.6 mM, 25 mM, about 50 mM, about 100 mM, about 150 mM, about 200 mM, about 250 mM, about 300 mM, about 350 mM, or about 400 mM of an amino acid.

[0171] In some aspects, the composition comprises a surfactant. The term "surfactant" as used herein refers to organic substances having amphipathic structures; namely, they are composed of groups of opposing solubility tendencies, typically an oil-soluble hydrocarbon chain and a water-soluble ionic group. Surfactants can be classified, depending on the charge of the surface-active moiety, into anionic, cationic, and nonionic surfactants. Surfactants are often used as wetting, emulsifying, solubilizing, and dispersing agents for various pharmaceutical compositions and preparations of biological materials. Pharmaceutically acceptable surfactants like polysorbates (*e.g.*, polysorbates 20 or 80); polyoxamers (*e.g.*, poloxamer 188); Triton; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (*e.g.,* lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUA® series (Mona Industries, Inc., Paterson, N.J.), polyethyl glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (*e.g.*, PLURONICS® PF68, etc.), can optionally be added to the compositions to reduce aggregation. In certain aspects, a composition comprises Polysorbate 20, Polysorbate 40, Polysorbate 60, or Polysorbate 80. Surfactants are particularly useful if a pump or plastic container is used to administer the composition. The presence of a pharmaceutically acceptable surfactant mitigates the propensity for the protein to aggregate. The compositions may comprise a polysorbate which is at a concentration ranging from between about 0.001% to about 1%, or about 0.001% to about 0.1%, or about 0.01% to about 0.1%. In other specific aspects, the compositions comprise a polysorbate which is at a concentration of 0.001%, or 0.002%, or 0.003%, or 0.004%, or 0.005%, or 0.006%, or 0.007%, or 0.008%, or 0.009%, or 0.01%, or 0.015%, or 0.02%.

[0172] In some aspects, the composition comprises other excipients and/or additives including, but not limited to, diluents, binders, stabilizers, lipophilic solvents, preservatives, adjuvants, or the like. Pharmaceutically acceptable excipients and/or additives may be used in the compositions provided herein. Commonly used excipients/additives, such as pharmaceutically acceptable chelators (for example, but not limited to, EDTA, DTPA or EGTA) can optionally be added to the compositions to reduce aggregation. These additives are particularly useful if a pump or plastic container is used to administer the composition.

[0173] In some aspects, the composition comprise a preservative. Preservatives, such as phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, phenylmercuric nitrite, phenoxyethanol, formaldehyde, chlorobutanol, magnesium chloride (for example, but not limited to, hexahydrate), alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof can optionally be added to the compositions at any suitable concentration such as between about 0.001% to about 5%, or any range or value therein. The concentration of preservative used in the compositions is a concentration sufficient to yield a microbial effect. Such concentrations are dependent on the preservative selected and are readily determined by the skilled artisan.

[0174] In some aspects, the composition is isotonic with human blood, wherein the compositions have essentially the same osmotic pressure as human blood. Such isotonic compositions will generally have an osmotic pressure from about 250 mOSm to about 350 mOSm. Isotonicity can be measured by, for example, using a vapor pressure or ice-freezing type osmometer. Tonicity of a composition is adjusted by the use of tonicity modifiers. "Tonicity modifiers" are those pharmaceutically acceptable inert substances that can be added to the composition to provide an isotonity of the composition. Tonicity modifiers suitable for the compositions provided herein include, but are not limited to, saccharides, salts and amino acids.

[0175] In certain aspects, the composition is a pyrogen-free composition which is substantially free of endotoxins and/or related pyrogenic substances. Endotoxins include toxins that are confined inside a microorganism and are released only when the microorganisms are broken down or die. Pyrogenic substances also include fever-inducing, thermostable substances from the outer membrane of bacteria and other microorganisms. Both of these substances can cause fever, hypotension and shock if administered to humans. Due to the potential harmful effects, even low amounts of endotoxins must be removed from intravenously administered pharmaceutical drug solutions. The Food & Drug Administration ("FDA") has set an upper limit of 5 endotoxin units (EU) per dose per kilogram body weight in a single one-hour period for intravenous drug applications (The United States Pharmacopeial Convention, Pharmacopeial Forum 26 (1):223 (2000)). When therapeutic proteins are administered in amounts of several hundred or thousand milligrams per kilogram body weight, as can be the case with proteins of interest (*e.g.*, antibodies), even trace amounts of harmful and dangerous endotoxin must be removed. In some aspects, the endotoxin and pyrogen levels in the composition are less than 10 EU/mg, or less than 5 EU/mg, or less than 1 EU/mg, or less than 0.1 EU/mg, or less than 0.01 EU/mg, or less than 0.001 EU/mg.

[0176] When used for in vivo administration, the composition described herein should be sterile. The composition may be sterilized by various sterilization methods, including sterile filtration, radiation, etc. In certain aspects, composition is filter-sterilized with a presterilized 0.22-micron filter. Sterile compositions for injection can be formulated according to conventional pharmaceutical practice as described in "Remington: The Science & Practice of Pharmacy", 21st ed., Lippincott Williams & Wilkins, (2005). Compositions comprising proteins of interest (*e.g.*, antibodies or TCRs or CARs) such as those disclosed herein, ordinarily will be stored in lyophilized form or in solution. It is contemplated that sterile compositions comprising proteins of interest (*e.g.*, antibody, TCR or CAR) are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having an adapter that allows retrieval of the composition, such as a stopper pierceable by a hypodermic injection needle. In certain aspects, a composition is provided as a pre-filled syringe.

[0177] In certain aspects, the composition is a lyophilized formulation. The term "lyophilized" or "freeze-dried" includes a state of a substance that has been subjected to a drying procedure such as lyophilization, where at least 50% of moisture has been removed.

[0178] Regardless of the route of administration selected, agents provided herein, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the provided herein, are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art.

Therapeutic Methods

[0179] In certain aspects, provided herein are methods of treating a disease or disorder comprising administering to a subject an antigen binding protein (*e.g.,* an antibody, a TCR and/or a CAR described herein, such as a fully human antibody, TCR or CAR). In some aspects, the antibody, a TCR and/or a CAR is an antibody, a TCR and/or a CAR obtained from or obtainable using the methods described herein (*e.g.*, using a non-human animal described herein).

[0180] In certain aspects, provided herein are methods of treating cancer in a subject comprising administering to the subject a pharmaceutical composition described herein (*e.g.,* a pharmaceutic composition comprising an antibody described herein, such as a fully human antibody, TCR or CAR described herein, obtained from the non-human animal as described herein). In some aspects, the methods described herein can be used to treat any cancerous or pre-cancerous tumor. Cancers that may be treated by methods and compositions described herein include, but are not limited to, cancer cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestine, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. Nonlimiting examples of various histological types of cancer include: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; and roblastoma, malignant; sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malig melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant;

dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; Hodgkin's disease; Hodgkin's lymphoma; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-Hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia.

[0181] In certain aspects, the antibody, TCR or CAR in the pharmaceutical composition administered to the subject has binding specificity for an epitope of a cancer-associated antigen (*e.g.,* an epitope expressed by the cancer being treated). Examples of cancer-associated antigens include, but are not limited to, adipophilin, AIM-2, ALDH1A1, alpha-actinin-4, alpha-fetoprotein ("AFP"), ARTC1, B-RAF, BAGE-1, BCLX (L), BCR-ABL fusion protein b3a2, beta-catenin, BING-4, CA-125, CALCA, carcinoembryonic antigen ("CEA"), CASP-5, CASP-8, CD274, CD45, Cdc27, CDK12, CDK4, CDKN2A, CEA, CLPP, COA-1, CPSF, CSNK1A1, CTAG1, CTAG2, cyclin D1, Cyclin-A1, dek-can fusion protein, DKK1, EFTUD2, Elongation factor 2, ENAH (hMena), Ep-CAM, EpCAM, EphA3, epithelial tumor antigen ("ETA"), ETV6-AML1 fusion protein, EZH2, FGF5, FLT3-ITD, FN1, G250/MN/CAIX, GAGE-1,2,8, GAGE-3,4,5,6,7, GAS7, glypican-3, GnTV, gp100/Pmel17, GPNMB, HAUS3, Hepsin, HER-2/neu, HERV-K-MEL, HLA-A11, HLA-A2, HLA-DOB, hsp70-2, IDO1, IGF2B3, IL13Ralpha2, Intestinal carboxyl esterase, K-ras, Kallikrein 4, KIF20A, KK-LC-1, KKLC1, KM-HN-1, KMHN1 also known as CCDC110, LAGE-1, LDLR-fucosyltransferaseAS fusion protein, Lengsin, M-CSF, MAGE-A1, MAGE-A10, MAGE-A12, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-C1, MAGE-C2, malic enzyme, mammaglobin-A, MART2, MATN, MC1R, MCSP, mdm-2, ME1, Melan-A/MART-1, Meloe, Midkine, MMP-2, MMP-7, MUC1, MUC5AC, mucin, MUM-1, MUM-2, MUM-3, Myosin, Myosin class I, N-raw, NA88-A, neo-PAP, NFYC, NY-BR-1, NY-ESO-1/LAGE-2, OA1, OGT, OS-9, P polypeptide, p53, PAP, PAX5, PBF, pml-RARalpha fusion protein, polymorphic epithelial mucin ("PEM"), PPP1R3B, PRAME, PRDX5, PSA, PSMA, PTPRK, RAB38/NY-MEL-1, RAGE-1, RBAF600, RGS5, RhoC, RNF43, RU2AS, SAGE, secernin 1, SIRT2, SNRPD1, SOX10, Sp17, SPA17, SSX-2, SSX-4, STEAP1, survivin, SYT-SSX1 or -SSX2 fusion protein, TAG-1, TAG-2, Telomerase, TGF-betaRII, TPBG, TRAG-3, Triosephosphate isomerase, TRP-1/gp75, TRP-2, TRP2-INT2, tyrosinase, tyrosinase ("TYR"), VEGF, WT1, XAGE-1b/GAGED2a. In some aspects, the antigen is a neo-antigen.

[0182] In certain aspects, provided herein are methods of treating a subject suffering from an infection, including a viral infection, a fungal infection, a bacterial infection, a helminth infection, or a protozoan infection, comprising administering to the subject a pharmaceutical composition described herein (*e.g.,* a pharmaceutic composition comprising an antibody, TCR or CAR described herein obtained from the non-human animals described herein). Nonlimiting examples of viral infectious diseases include HPV, HBV, hepatitis C Virus (HCV), retroviruses such as human immunodeficiency virus (HIV-1 and HIV-2), herpes viruses such as Epstein Barr Virus (EBV), cytomegalovirus (CMV), HSV-1 and HSV-2, and influenza virus. A nonlimiting example of parasitic infection is malaria. Nonlimiting examples of bacterial, fungal and other pathogenic diseases include *Aspergillus, Brugia, Candida, Chlamydia, Coccidia, Cryptococcus, Dirofilaria, Gonococcus, Histoplasma, Leishmania, Mycobacterium, Mycoplasma, Paramecium, Pertussis, Plasmodium, Pneumococcus, Pneumocystis, Rickettsia, Salmonella, Shigella, Staphylococcus, Streptococcus, Toxoplasma* and *Vibriocholerae.* Exemplary species include *Neisseria gonorrhea, Mycobacterium tuberculosis, Candida albicans, Candida tropicalis, Trichomonas vaginalis, Haemophilus vaginalis,* Group B *Streptococcus sp., Microplasma hominis, Hemophilus ducreyi, Granuloma inguinale, Lymphopathia venereum, Treponema pallidum, Brucella abortus. Brucella melitensis, Brucella suis, Brucella canis, Campylobacter fetus, Campylobacter fetus intestinalis, Leptospira pomona, Listeria monocytogenes, Brucella ovis, Chlamydia psittaci, Trichomonas foetus, Toxoplasma gondii, Escherichia coli, Actinobacillus equuli, Salmonella abortus ovis, Salmonella abortus equi, Pseudomonas aeruginosa, Corynebacterium equi, Corynebacterium pyogenes, Actinobaccilus seminis, Mycoplasma bovigenitalium, Aspergillus fumigatus, Absidia ramosa, Trypanosoma equiperdum, Babesia caballi, Clostridium tetani, Clostridium botulinum;* or, a fungus, such as, *e.g., Paracoccidioides brasiliensis;* or other pathogen, *e.g., Plasmodium falciparum.*

[0183] In certain aspects, the antibody, TCR or CAR in the pharmaceutical composition administered to the subject has binding specificity for an epitope of an antigen expressed by an infectious pathogen (*e.g.,* an epitope expressed by the infectious pathogen being treated).

[0184] In some aspects, provided herein is a method of treating an inflammatory disease, skin or organ transplantation rejection, graft-versus-host disease (GVHD), or autoimmune diseases, comprising administering to a subject a pharmaceutical composition described herein (*e.g.,* a pharmaceutic composition comprising an antibody, TCR or CAR described herein obtained from the non-human animals described herein). Examples of autoimmune diseases include, for example, glomerular nephritis, arthritis, dilated cardiomyopathy-like disease, ulceous colitis, Sjogren syndrome, Crohn disease, systemic erythematodes, chronic rheumatoid arthritis, multiple sclerosis, psoriasis, allergic contact dermatitis, polymyosiis, pachyderma, periarteritis nodosa, rheumatic fever, vitiligo vulgaris, insulin dependent diabetes mellitus, Behcet disease, Hashimoto disease, Addison disease, dermatomyositis, myasthenia gravis, Reiter syndrome, Graves' disease, anaemia perniciosa, Goodpasture syndrome, sterility disease, chronic active hepatitis, pemphigus, autoimmune thrombopenic purpura, and autoimmune hemolytic anemia, active chronic hepatitis, Addison's disease, anti-phospholipid syndrome, atopic allergy, autoimmune atrophic gastritis, achlorhydra autoimmune, celiac disease, Cushing's syndrome, dermatomyositis, discoid lupus, erythematosis, Goodpasture's syndrome, Hashimoto's thyroiditis, idiopathic adrenal atrophy, idiopathic thrombocytopenia, insulin-dependent diabetes, Lambert-Eaton syndrome, lupoid hepatitis, some cases of lymphopenia, mixed connective tissue disease, pemphigoid, pemphigus vulgaris, pernicious anema, phacogenic uveitis, polyarteritis nodosa, polyglandular autosyndromes, primary biliary cirrhosis, primary sclerosing cholangitis, Raynaud's syndrome, relapsing polychondritis, Schmidt's syndrome, limited scleroderma (or crest syndrome), sympathetic ophthalmia, systemic lupus erythematosis, Takayasu's arteritis, temporal arteritis, thyrotoxicosis, type b insulin resistance, ulcerative colitis and Wegener's granulomatosis.

[0185] In certain aspects, the antibody, TCR or CAR in the pharmaceutical composition administered to the subject has binding specificity for the target of an autoreactive T cell in the disease being treated (*e.g.,* an epitope targeted by autoreactive T cells in an autoimmune disease). Exemplary proteins include targeted by autoreactive T cells include, for example, p205, insulin, thyroid-stimulating hormone, tyrosinase, TRP1, and myelin.

[0186] The pharmaceutical compositions described herein may be delivered by any suitable route of administration, including orally, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually. In certain aspects the pharmaceutical compositions are delivered generally (*e.g.*, via oral or parenteral administration).

[0187] Actual dosage levels of the active ingredients in the pharmaceutical compositions described herein may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

[0188] The selected dosage level will depend upon a variety of factors including the activity of the particular agent employed, the route of administration, the time of administration, the rate of excretion or metabolism of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[0189] In some aspects, the CAR and/or TCR described here are used for T cell based therapy. For example, in certain aspects, T cells expressing a CAR and/or TCR described herein are administered to a subject to induce a T cell based immune response in the subject. Methods useful in T cell based therapy is described in, for example, in Schumacher Nat. Rev. Immunol. 2:512-519 (2002) and Bitton et al., Frontiers in Bioscience 4:d386-393

[0190] (1999).

[0191] In some aspects, provided herein is a method of inducing an immune response (*e.g.,* a

T cell based immune response) in a subject. In some aspects, the method includes administering to the subject a cell (*e.g.,* a human T cell, such as a CD4 T cell or a CD8 T cell) expressing a CAR or TCR described herein.

[0192] In some aspects, the subject is a subject in need thereof. In some aspects, the subject is a subject with cancer or a subject infected with a pathogen. In such aspects, the peptide in the peptide/MHC complex recognized by the CAR or TCR is a peptide of a cancer antigen or a peptide from an antigen expressed by an infectious pathogen.

[0193] In some aspects, provided herein is a method of inhibiting an immune response in a subject. In some aspects, the method include administering to the subject a regulatory T cell (*e.g.,* a CD4$^+$, CD-25$^+$ and Foxp3$^+$ regulatory T cell or a Treg17 T cell) expressing a CAR or TCR described.

[0194] In some aspects, the subject is a subject in need thereof, e.g., a subject with an autoimmune disease. In such aspects, the T cell is a regulatory T cell (*i.e.,* a suppressor T cell) and the peptide in the peptide/MHC complex recognized by the TCR or CAR is a self-antigen to which the subject is undergoing an autoimmune response.

Nucleic Acid Molecules

[0195] Provided herein are nucleic acid molecules that encode the antibodies, TCRs or CARs described herein and/or portions of antibodies, TCRs and CARs described herein. In some embodiments, the nucleic acid encodes a variable domain of an antibody, TCR or CAR described herein. The nucleic acid molecules may be present, for example, in whole cells, in a cell lysate, or in a partially purified or substantially pure form.

[0196] In certain aspects, provided herein are nucleic acids encoding an antibody, TCR and/or CAR polypeptide described herein or a portion thereof. The nucleic acids may be present, for example, in whole cells, in a cell lysate, or in a partially purified or substantially pure form. Nucleic acids described herein can be obtained using standard molecular biology techniques. For example, nucleic acid molecules described herein can be cloned using standard PCR techniques or chemically synthesized. For nucleic acids encoding CARs, TCRs or antibodies expressed by hybridomas, cDNAs encoding each chain of the antibody, TCR or CAR made by the hybridoma can be obtained by standard PCR amplification or cDNA cloning techniques.

[0197] In certain aspects, provided herein are vectors that contain the nucleic acid molecules described herein. As used herein, the term "vector," refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby be replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors").

[0198] In certain aspects, provided herein are cells that contain a nucleic acid described herein (*e.g.,* a nucleic acid encoding an antibody, TCR or CAR described herein or a portion thereof). The cell can be, for example, prokaryotic, eukaryotic, mammalian, avian, murine and/or human. In certain aspects the nucleic acid described herein is operably linked to a transcription control element such as a promoter. In some aspects the cell transcribes the nucleic acid described herein and thereby expresses an antibody, antigen binding fragment thereof or polypeptide described herein. The nucleic acid molecule can be integrated into the genome of the cell or it can be extrachromasomal.

[0199] Nucleic acid molecules provided herein can be obtained using standard molecular biology techniques. For example, nucleic acid molecules described herein can be cloned using standard PCR techniques or chemically synthesized.

[0200] For antibodies and CAR nucleic acids described herein, once DNA fragments encoding a V_H and V_L segments are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a V_L- or V_H-encoding DNA fragment is operably linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operably linked", as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

[0201] The isolated DNA encoding the heavy chain variable region can be converted to a full-length heavy chain gene by operably linking the heavy chain variable region DNA to another DNA molecule encoding heavy chain constant regions (*e.g.,* CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see *e.g.,* Kabat, E. A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, or Lefranc, The Immunoglobulin Handbook, London: Academic Press 2001) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region. For a Fab fragment heavy chain gene, the V_H-encoding DNA can be operably linked to another DNA molecule encoding only the heavy chain CH1 constant region.

[0202] The isolated DNA encoding the light chain variable region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operably linking the light chain variable region encoding DNA to another DNA molecule encoding a light chain constant region. The sequences of human light chain constant region genes are known in the art (see *e.g.,* Kabat, E. A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, or Lefranc, The Immunoglobulin Handbook, London: Academic Press 2001) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region.

EXAMPLES

[0203] The invention will be further illustrated by the following nonlimiting examples.. The Examples do not include detailed descriptions of conventional methods that would be well known to those of ordinary skill in the art (molecular cloning techniques, *etc.*). Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is indicated in Celsius, and pressure is at or near atmospheric.

Example 1: Generation of Mice Expressing Human TdT

[0204] Mice comprising a human TdT gene, either as random transgene or targeted to the immunoglobulin kappa locus, are made using VELOCIGENE® genetic engineering technology (see, *e.g.,* US Pat. No. 6,586,251 and Valenzuela, D.M., et al. (2003) High-throughput engineering of the mouse genome coupled with high-resolution expression analysis. Nat. Biotech. 21(6): 652-659), wherein human sequences derived from BAC libraries using bacterial homologous recombination are used to make large targeting vectors (LTVECs) comprising genomic fragments of human TdT locus and, in the case of TdT targeted to the IgK locus, flanked by targeting arms to target the LTVECs to the IgK locus in mouse ES cells. LTVECs are linearized and electroporated into a mouse ES cell line according to Valenzuela et al. ES cells are screened by TAQMAN® to determine either gene copy number (for randomly integrated transgenes) or correct targeting to the IgK locus.

[0205] Alternatively, a short isoform human TdT (TdTS) cDNA is synthesized de novo (Blue Heron Bio) and incorporated into a targeting for introduction into ES cells as described above.

[0206] Targeted ES cell clones are introduced into 8-cell stage (or earlier) mouse embryos by the VELOCIMOUSE® method (Poueymirou, W.T. et al. (2007). F0 generation mice fully derived from gene-targeted embryonic stem cells allowing immediate phenotypic analyses. Nat. Biotech. 25: 91-99). VELOCIMICE® (F0 mice fully derived from the donor ES cell) bearing human TdT locus are identified by screening by TAQMAN® in a gain of human allele assay (Valenzuela et al.). F0 pups are genotyped and bred to homozygosity. Mice homozygous for human TdT locus are made and phenotyped. As specifically described in Example 1 below, mice comprising human TdTS and unrearranged human variable light and heavy chain loci are generated by introducing human TdTS random transgene or targeting human TdTS to IgK locus in VELOCIMMUNE® mice comprising a functional ectopic mouse Adam6 gene. However, at least for the randomly introduced human TdTS transgene, such animals can also be generated by first generating ES cells comprising a human TdTS as described below, generating mice therefrom, and breeding mice comprising randomly integrated human TdTS locus with VELOCIMMUNE® mice comprising a functional ectopic mouse Adam6 gene.

Example 1.1. Generation of Transgene Expressing Short Isofom of Human TdT (TdTS) Under Control of Mouse Rag Regulatory Elements (Rag-TdT tg)

[0207] Briefly, a large targeting vector (LTVEC), shown in detail in **FIG. 1,** was constructed from mouse and human BAC clones in which the mouse Rag2 gene (from the ATG start codon in exon 3 to the TGA stop codon in exon 3 was replaced with the human Terminal deoxynucleotidyl Transferase (TdT or DNTT) gene encoding only the short isoform, TdTS (from the ATG start codon in exon 1 to ~0.5kb 3' of the polyA signal in exon 13). The RNA splice sites of TdT exons 7 and 12 were mutated to prevent expression of the long isoforms (TdTL1 and L2). In the same LTVEC, the mouse Rag1 gene from the ATG start codon in exon 2 to the TAA stop codon in exon 2 was replaced with the coding sequence of the enhanced green fluorescent protein (EGFP) and the LacZ 3'UTR-polyA signal. The LTVEC contains 130kb of upstream regulatory sequences 5' of the mouse Rag2 gene and 8.8kb of upstream regulatory sequences 3' of the mouse Rag1 gene, as well as the 5.6kb Rag2-Rag1 intergenic region.

[0208] The LTVEC was constructed from mouse BAC clone RP23-374f10 (Invitrogen/Life Technologies) containing the Rag2 and Rag1 genes, and human BAC clone RP11-1070o2 (Invitrogen/Life Technologies) containing the TdT gene using standard molecular biology and recombineering techniques such as PCR, restriction digestion/ligation, Gibson Isothermal Assembly, CRISPR/Cas9, bacterial homologous recombination, *etc.* The final LTVEC contains, from 5' to 3': (1) a Loxp-pgkp-em7-neo-loxp cassette for selection in ES cells or bacteria, (2) 134,069bp of mouse genomic sequence beginning 129,440bp upstream of Rag2 exon 1 and ending 25bp from the start of Rag2 exon 3 (mouse genome coordinates 2:101,495,278-101,629,347 based on GRCm38 assembly), (3) 34,573bp of human TdT genomic sequence beginning at the ATG start codon in exon 1 and ending 514bp 3' of the polyA signal (human genome coordinates 10:96,304,498-96,339,063 based on GRCh38 assembly); within the human TdT gene, the splice donor site of exon 7 is deleted and replaced by a NotI site to prevent expression of the TdTL2 isoform, and the splice acceptor site of exon 12 is deleted to prevent expression of the TdTL1 isoform (detailed description below), (4) AsiSI site; (5) 10,742bp of mouse genomic sequence (GRCm38 genome coordinates 2:101,630,931-101,641,672) containing the 3'UTR of Rag2 exon 3 (1599bp), the 5753bp Rag2/Rag1 intergenic region, and the 3'UTR of Rag1 exon 2 (3390bp), (6) FseI site, (7) 1,068bp on the minus strand containing the 249bp polyA signal of LacZ and the 793bp CDS of EGFP, (8) 13,459bp of mouse genomic sequence (GRCm38 coordinates 2:101,644,793-101,658,251) with Rag1 on the minus strand, beginning at the ATG start codon in Rag1 exon 2 and ending 8,750bp 3' of the transcription start site of Rag1, and (9) Em7-CM cassette for selection in bacteria (see **FIG. 1).**

[0209] In detail, the cloning steps to create two modifications of the TdT gene to prevent alternative splicing using TdT exons 7 and 12 (which are used to make the long isoforms TdTL2 and TdTL1, respectively) while still allowing splicing of the transcript encoding the short isoform TdTS were constructed from BAC clone RP11-1070o2 using BHR and ligation:

1. (1) 13bp including the splice donor site of exon 7 (GTCGGGTCGTGGT (SEQ ID NO: 1), splice donor underlined) were deleted and replaced by a NotI site (GCGGCCGC (SEQ ID NO:2)). This created an overlapping SacII site (CCGCGG (SEQ ID NO:3)), and
2. (2) The 2bp splice acceptor site of exon 12 was deleted.

[0210] The final LTVEC is depicted in **FIG. 1,** with the approximate positions of various sequence junctions indicated in the figure. The junctions are also summarized in Table 1 below.

Table 1: Sequence Junctions of Rag-TdT Tg LTVEC

Junction	SEQ ID NO	Sequence
1 (mouse Rag2/human TdT)	4	TATTGCGTTTTTTTAATCCTTTCAGATAAAAGACCTA TTCACAATCAAAA/ ATGGATCCACCACGAGCGTCCC ACTTGAGCCCTCGGAAGAAGAGACCCC
2 (human TdT/AsiS1/mouse Rag2)	5	GCCCTGGCTGAGGGAAATTTTGGAACTCCCAGGC TCCAGACCCATTCTTT/ GCGATCGC/ TTTAGCAAA AGCCCCTCAGACTCAGGTATATTGCTCTCT GAATCTACTTT
3 (mouse Rag1/Fse1/EGFP)	6	CCAAAGGAAAACACATTGGCAAATACCAACTTCTATG TGGAGATCCTAT/ GGCCGGCC/ GGGGATCCAGACATGA TAAGATACATTGATGAGTTTGGACAAACCACAAC
4 (EGFP/mouse Rag1)	7	TCGACCAGGATGGGCACCACCCCGGTGAACAGCTC CTCGCCCTTGCTCAC/ CATGTTGGCTAAGC TACCTGGGAACAATGGGGGGGGGGGGGGA GTCAAG

[0211] The final LTVEC was linearized and electroporated into VELOCIMMUNE® ES cells that comprise a functional ectopic mouse Adam6 gene (see, *e.g.,* U.S. Patent No. 8,642,835). After selection with Neo, ES cells were screened by TAQMAN® to determine copy number of the transgene. ES cells comprising a single copy, two copies, or multiple copies of the human Rag-TdT transgene were obtained.

[0212] The integration site of the Rag-TdT transgene is determined via methods known in the art. For instance, the integration site is determined using low coverage paired-end sequencing of the whole mouse genome (Sequencing library - Nextera DNA Library Preparation, Illumina; Sequence - Miseq, Illumina). For example, in one instance it was determined that Rag-TdT transgene integrated as two tandem head to tail copies between coordinates 41130492 and 41130502 on chromosome 1 (coordinates in GRCm38/mm10 Assembly), without disrupting any coding regions.

Example 1.2. Generation of Targeted Immunoglobulin Kappa Locus Insertion of Short Isoform of Human TdT (TdTS) Under Control of Mouse Rag Regulatory Elements (Rag-TdT IgK)

[0213] In order to generate a mouse comprising human TdT under control of the Rag regulatory elements on the mouse immunoglobulin kappa locus, mouse IgK homology arms for recombination in ES cells were added to the 5' and 3' ends of the construct that is generated as described in Example 1.1 and **FIG. 1.** In general, the mouse IgK arms are added by: (1) modifying the mouse IgK BAC to by bacterial homologous recombination to insert a selection cassette (*e.g.,* Hyg, Neo, *etc.*) flanked by I-CeuI and PI-SceI restriction enzyme sites, and (2) inserting the TdT construct into the mouse IgK BAC by I-CeuI and PI-SceI ligation. The final LTVEC depicted in **FIG. 2.**

[0214] This final LTVEC contains, from 5' to 3': (1) a Spec cassette for selection in bacteria, (2) a 28591bp 5' mouse homology arm (GRCm38 genome coordinates 6:70,725,823-70,754,415) containing the IgKc gene, the IgK 3' enhancer, and the 3' IgK recombining sequence (RS); the mouse arm ends ~2.6kb 3' of the RS, (3) PI-SceI site, (4) a loxp-UbCp-em7-hyg-loxp cassette for selection in ES cells or bacteria, (5) the construct described above in Example 1.1 and FIG. 1 containing the Rag2 promoter-human TdTS and Rag1-EGFP genes, (6) I-CeuI site, (7) a 44,900bp 3' mouse IgK homology arm (GRCm38 genome coordinates 6:70,754,508-70,799,678), and (8) CM cassette for selection in bacteria.

[0215] The final LTVEC is depicted in **FIG. 2,** with the approximate positions of various sequence junctions indicated in the figure. The junctions are also summarized in Table 2 below.

Table 2: Sequence Junctions of Rag-TdT IgK LTVEC

Junction	SEQ ID NO	Sequence
1. (mouse IgK/PI-Sce1/loxp-Ub-Hyg cassette)	**8**	CATCCTTACATCTTTGTCATCCCCTGTATCAACA TGGAAAGGCATTAATG/ATCTATGTCGGGTGCGG AGAAAGAGGTAATGAAATGGCA/ACCGGTATAA CTTCGTATAATGTATGCTATACGAAGTTATATG CATGGCC
2. (loxp-Ub-Hyg cassette/mouse Rag2)	**9**	TTCGTATAATGTATGCTATACGAAGTTATGTC GACCTCGAGGGGGGGCCC/ACCTCCAGC TGCCTTACAGAAAAGCAAATGCTTGCTTGCA ACAATCACCT
3. (mouse Rag2/human TdTS)	**10**	TATTGCGTTTTTTTAATCCTTTCAGATAAAA GACCTATTCACAATCAAAA/ATGGATCCACC ACGAGCGTCCCACTTGAGCCCTCGGAAGAA GAGACCCC
4. (human TdTS/AsiS1/mouse Rag2)	**11**	GCCCTGGCTGAGGGAAATTTTGGAACTCCCAG GCTCCAGACCCATTCTTT/GCGATCGC/TTTAG CAAAAGCCCCTCAGACTCAGGTATATTGCTCT CTGAATCTACTTT
5. (mouse Rag1/Fse1/EGFP)	**12**	CCCAAAGGAAAACACATTGGCAAATACCAA CTTCTATGTGGAGATCCTAT/GGCCGGCC/GG GGATCCAGACATGATAAGATACATTGATGAG TTTGGACAAACCACAAC
6. (EGFP/mouse Rag1)	**13**	TCGACCAGGATGGGCACCACCCCGGTGAACA GCTCCTCGCCCTTGCTCAC/CATGTTGGCTAA GCTACCTGGGAACAATGGGGGGGGGGGGGGG AGTCAAG
7. (mouse Rag1/I-CeuI/mouse IgK)	**14**	ACCTCTGCTGTGTCTGCAAGTTTGGCTTGTTC CTGCTTCTGATTTTTGGG/TCTAGACCCCCGGG CTCGATAACTATAACGGTCCTAAGGTAGCGA
		CTCGAG/CATAACCACTTTCCTGCTATGGATCT CTTAAATATCCCCCAAACCCCAAC

Junction	SEQ ID NO	Sequence
		GTTAAATATCCGCCAAAGGCCAAG

[0216] The resulting LTVEC was linearized and electroporated into VELOCIMMUNE® ES cells that comprise a functional ectopic mouse Adam6 gene (see, *e.g.,* U.S. Patent No. 8,642,835). After selection for Hyg-resistance, ES cells were screened by TAQMAN® modification of allele assay (Valenzuela et al, *supra*) to identify correctly targeted clones.

Example 1.3. Generation of Random Transgenic and Targeted Immunoglobulin Kappa Locus Insertion of Short Isoform of Human TdT (TdTS), Both Under Control of Mouse Immunoglobulin Heavy Chain Intronic Enhancer (Eμ) and Mouse IgV$_H$1-72 Promoter (mIgH-Eμ-V$_H$1-72-TdT tg and mIgH-Eμ-V$_H$1-72-TdT IgK, Respectively)

[0217] The same human TdTS gene as used to make the Rag-TdT in Examples 1.1 and 1.2 (*i.e.,* from ATG start codon to about 514 bp 3' of the polyA signal), was placed under the control of the 689bp mouse Eμ enhancer and 303bp mouse IgV$_H$1-72 promoter. This construct was either randomly integrated into the mouse genome or targeted to the immunoglobulin K (IgK) locus. For targeted integration, the gene was inserted between the same 5' and 3' mouse IgK homology arms as were used to make the LTVEC in Example 1.2.

[0218] Specifically, the final LTVEC contains, from 5' to 3' **(FIG. 3):** (1) a Spec cassette for selection in bacteria, (2) a 28591bp 5' mouse homology arm (GRCm38 genome coordinates 6:70,725,823-70,754,415) containing the IgK constant (IgKC) gene, the IgK 3' enhancer, and the 3' IgK recombining sequence (RS); the mouse arm ends ~2.6kb 3' of the RS, (3) I-CeuI site, (4) loxp-UbCp-em7-hyg-loxp cassette in reverse orientation for selection in ES cells or bacteria, (5) the same 34,573bp human TdTS gene used in Examples above in reverse orientation, (6) the 303bp mouse IgHV1-72 promoter in reverse orientation (GRCm38 genome coordinates 12:115,758,417-115,758,719), (7) The 689bp mouse Eμ enhancer (EcoRI-XbaI fragment, GRCm38 genome coordinates 12:113,427,284-113,427,972) in reverse orientation, (8) PI-SceI site, (9) a 44,900bp 3' mouse IgK homology arm (GRCm38 genome coordinates 6:70,754,508-70,799,678), and (10) CM cassette for selection in bacteria.

[0219] The approximate positions of the specific sequence junctions in the final vector are depicted in **FIG. 3,** and their sequences indicated in Table 3 below.

Table 3: Sequence Junctions of mIgH-Eμ-V$_H$1-72-TdT IgK LTVEC

Junction	**SEQ ID NO**	**Sequence**
1. (mouse/ICeu1/loxp-Ub-Hyg cassette)	15	CATCCTTACATCTTTGTCATCCCCTGTAT CAACATGGAAAGGCATTAATG/TCGCTA CCTTAGGACCGTTATAGTTA/GGCCCCCCCTCGA GGTCGACATAACTTCGTATAGCATACATTATACGAAG
2. (loxp-Ub-Hyg cassette/human TdT)	16	GGCCATGCATATAACTTCGTATAGCAT ACATTATACGAAGTTATACCGGT/AAA GAATGGGTCTGGAGCCTGGGAGTTCCA AAATTTCCCTCAGCCAGGGC
3. (human TdT/mouse IgHV1-72)	17	CGGGGTCTCTTCTTCCGAGGGCTCAAGT GGGACGCTCGTGGTGGATCCAT/GGTGAG GTCCTGTGTGCTCAGTAACTGTAAAGAGA ACAGTGATCTCATGT
4. (mouse Eμ/PI-SceI/mouse IgK)	18	TAGTTTCCCCAAACTTAAGTTTATCGACTTCTA AAATGTATTTAGAATTC/TGCCATTTCATTACC TCTTTCTCCGCACCCGACATAGATAAAGCTT/CA TAACCACTTTCCTGCTATGGATCTGTTAAATAT CCGCCAAAGGCCAAG

[0220] The resulting LTVEC was linearized and electroporated into VELOCIMMUNE$^{®}$ ES cells that comprise a functional ectopic mouse Adam6 gene (see, *e.g.,* U.S. Patent No. 8,642,835). After selection for Hyg-resistance, ES cell clones were screened by TAQMAN$^{®}$ for correct targeting to the mouse IgK locus (for mIgH-Eμ-V_H1-72-TdT IgK) or for transgene copy number (for mIgH-Eμ-V_H1-72-TdT tg).

Example 1.4. Generation of Targeted Immunoglobulin Kappa Locus Insertion as well as Transgenic Human TdTS from TdTS cDNA

[0221] Alternatively, a TdTS cDNA is synthesized de novo (Blue Heron Bio) as a 3682bp DNA fragment and incorporated into a targeting vector for introduction into ES cells. The targeting vector contains, from 5' to 3', a PI-SceI site, the 689bp mouse IgH intronic enhancer (EcoRI-XbaI fragment), the 303bp mouse VH1-72 promoter, the 1530bp CDS of human TdTS (NCBI RefSeq NM_004088) with the 735bp intron 2 retained between exons 2 and 3 for intron-mediated enhancement of expression, the 340bp human TdT 3' UTR/polyA signal, NotI and SalI restriction enzyme sites for ligating in a loxp-neo-loxp cassette, and an I-CeuI site. The vector was inserted between the same 5' and 3' mouse IgK homology arms as were used to make the LTVEC in Example 1.2 and either targeted to the IgK locus or randomly integrated into the mouse genome.

[0222] The resulting LTVEC is linearized and electroporated into VELOCIMMUNE® ES cells that comprise a functional ectopic mouse Adam6 gene (see, *e.g.,* U.S. Patent No. 8,642,835). After selection for Hyg-resistance, ES cell clones are screened by TAQMAN® for correct targeting to the mouse IgK locus (for IgK targeted version) or for transgene copy number (for transgenic version).

Example 1.5. Mice Expressing Human TdTS

[0223] As described above, once correctly targeted ES cells are produced, they are introduced into 8-cell stage (or earlier) mouse embryos by the VELOCIMOUSE® method, screened in a gain of allele assay, and subsequently bred to homozygosity. Heterozygous or homozygous animals express human TdTS as well as antibodies comprising human variable light and heavy chain domains and mouse constant regions (as these mice comprise human immunoglobulin variable light and heavy gene segments at the endogenous IgK and IgH loci, respectively: VELOCIMMUNE® mice).

[0224] Several versions of human TdTS mice were generated and tested, and those included random transgenic and IgK targeted TdTS both under the control of Rag promoter and Eμ-V_H1-72 regulatory elements. Also included were versions with one, two, or several copies of the TdTS transgene; as well as versions generated from genomic TdT and cDNA TdT sequences. The remaining examples demonstrate data obtained with mice comprising a transgene of Rag-genomic TdTS (tandem insertion of two copies on chromosome 1, as described in Example 1.1 above) and Eμ-V_H1-72-genomic TdTS targeted to the IgK locus (Example 1.3).

[0225] First, mice are tested for expression of TdT. PT-PCR was used for amplify TdT transcripts from bone marrow of either VELOCIMMUNE® control, VELOCIMMUNE® + Rag-genomic TdTS transgene, or VELOCIMMUNE® + Eμ-V_H1-72-genomic TdTS targeted to the IgK locus mice. Total RNA was used for reverse transcription by SUPESCRIPT® III Reverse Transcriptase (Life Technologies) using Oligo-dT primer. PCR was conducted using SsoAdvanced™ Universal SYBR® Green Supermix, with primers for either Beta Actin (control), primers designed to amplify exons 1-2, primers designed to amplify exons 4-6, primers designed to amplify exons 7-9, and primers designed to amplify exons 9-11. As shown in **FIG. 4,** presence of human TdT exons was detected in both versions of the mice, while absent from

VELOCIMMUNE® control mice.

Example 2. Human Immunoglobulin Kappa Junctional Diversity in Mice Comprising Human TdTS

[0226] To assess immunoglobulin repertoire sequence diversity in various VELOCIMMUNE® human TdTS mouse models described above in Example 1, IgK sequences were amplified by 5' RACE from spleens of various mice with mIgK constant primer and sequenced using Illumina MiSeq.

[0227] Specifically, splenic B cells were positively enriched from total splenocytes by magnetic cell sorting using anti-CD19 (mouse) magnetic beads and MACS® columns (Miltenyi Biotech). Total RNA was isolated from the purified splenic B cells using an RNeasy Plus RNA isolation kit (Qiagen) according to manufacturer's instructions. Reverse transcription was performed to generate cDNA containing Igκ constant region sequence, using a SMARTer™ RACE cDNA Amplification Kit (Clontech) and an Igκ specific primer **(Table 4).** During this process, a DNA sequence, which is reverse compliment to 3' of primer PE2-PIIA, was attached to the 3' end of the newly synthesized cDNAs. Purified Igκ specific cDNAs were then amplified by the 1st round PCR using the PE2-PIIA primer and an Igκ constant specific primer listed in **Table 4.** PCR products between 450-700bp were isolated using Pippin Prep (SAGE Science). These products were further amplified by a 2nd round PCR using primers listed in **Table 4** ("XXXXXX" represents a 6bp index sequences to enable multiplexing samples for sequencing). PCR products between 400bp-700bp were isolated, purified, and quantified by qPCR using a KAPA Library Quantification Kit (KAPA Biosystems) before loading onto a Miseq sequencer (Illumina) for sequencing using Miseq Reagent Kits v3 (600 cycles).

Table 4: Primers used in library preparation for Igk repertoire sequencing

RT primers	**IgK (SEQ ID NO:19)**	5'- AAGAAGCACACGACTGAGGCAC- 3'
1st round PCR primers	**IgK Constant (SEQ ID NO:20)**	5′ - ACACTCTTTCCCTACACGACGCTCTTCCGATCT GGAAGATGGATACAGTTGGTGC - 3′
	PE2-PIIA (SEQ ID NO:21)	5′ - GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AAGCAGTGGTATCAACGCAGAGT - 3′
2nd round PCR Primers	**Forward (SEQ ID NO:22)**	5′ - AATGATACGGCGACCACCGAGATCTACACXXXX XX ACACTCTTTCCCTACACGACGCTCTTCCGATCT- 3′

RT primers	**IgK (SEQ ID NO:19)**	5'- AAGAAGCACACGACTGAGGCAC- 3'
	Reverse (SEQ ID NO:23)	5′ - CAAGCAGAAGACGGCATACGAGATXXXXXX GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3′

[0228] For bioinformatics analysis, raw Illumina sequences were de-muliplexed and filtered based on quality, length and perfect match to kappa constant region primer. Overlapping paired-end reads were merged and analyzed using custom in-house pipeline. The pipeline used local installation of IgBLAST (NCBI, v2.2.25+) to align rearranged light chain sequences to human germline V and J gene database. Rearrangements were considered productive if no stop codons were detected and VJ junction was in-frame with J segment. Otherwise rearrangements were considered nonproductive and excluded from analysis.

[0229] CDR3 sequences were extracted using International Immunogenetics Information System (IMGT) boundaries. Junctional region between annotated V and J segments was classified as P and N nucleotides. Region with N/P additions was extracted from each sequence and its length calculated. Diversity of antibody repertoire was calculated by analyzing unique clonotypes. Sequence diversity was defined as a number of unique CDR3 sequences in randomly chosen 10,000 reads.

[0230] FIG. 5 shows that up to 2-fold increase in the number of unique CDR3 amino acid sequences was detected in human TdTS mouse models compared with VELOCIMMUNE® mice that did not comprise human TdTS. Increased CDR3 diversity was also observed on the nucleotide level (data not shown). **FIG. 5** only shows data obtained with mice comprising two copies of transgene of Rag-genomic TdTS (Rag TdT Tg) and Eμ-V_H1-72-genomic TdTS targeted to the IgK locus (mIgH- Eμ-V_H1-72 TdT IgK) (both homozygous and heterozygous versions), while similar data was obtained from other versions of the mice (not shown).

Example 3. Increase in Non-Germline Additions in Mice Comprising Human TdTS

[0231] Percentage of non-germline nucleotide additions in CDR3 (which consists of parts of both Vκ and Jκ gene segments) was also determined from Next Generation Sequencing described in Example 2 above.

[0232] As depicted in **FIG. 6,** about 45% of humanized kappa light chain in B cells were shown to have non-germline additions in both versions of humanized TdTS mice as compared to about 10% in VELOCIMMUNE® mice that comprise a functional ectopic mouse Adam6 gene. Sequence analysis of immunoglobulin light chains from spleen showed 0 to 8 non-template additions in light chains of human TdTS mice (8 in the figure includes sequences with 8 or more non-template additions).

Example 4. Human Light Chain CDR3 Lengths in Immunoglobulins Obtained from Human TdTS mice

[0233] CDR3 sequences were extracted using International Immunogenetics Information System (IMGT) boundaries. Non-template nucleotides were determined based on known light chain V and J sequences.

[0234] As depicted in **FIG. 7A,** increased non-template additions observed in the two versions of the human TdTS mice described in Examples 2 and 3 above led to increase in kappa light chain CDR3 length compared to control (VELOCIMMUNE® mice that comprise a functional ectopic mouse Adam6 gene). As depicted in **FIG. 7B,** sequence analysis revealed no extensive exonuclease activity affecting 5' J trimming rates in Rag-TdTS mice (only data for heterozygous mice is depicted here) as compared to control (VELOCIMMUNE® mice that comprise a functional ectopic mouse Adam6 gene).

Example 5. Human Light Chain Vκ and Jκ Gene Segment Usage in Human TdTS Mice

[0235] As depicted in **FIGs. 8A and 8B,** introduction of human TdTS in the two versions of the mice described un Examples 2 and 3 above did not significantly alter the usage of either Vκ gene segments or Jκ gene segments compared to VELOCIMMUNE® mice that comprise a functional ectopic mouse Adam6 gene.

Example 6. Junctional Diversity at Light Chain Lambda Immunoglobulin Locus and Other Rearranging Loci in Mice Comprising Human TdTS

[0236] In addition to human immunoglobulin kappa locus, antigen-receptor diversity in other loci from B (λ light chain, heavy chain) and T (α/β) lymphocytes can be investigated.

[0237] For example, when C λ1-containing lambda light junctional diversity in VELOCIMMUNE® mice that comprise a functional ectopic mouse Adam6 gene was compared to the same VELOCIMMUNE® mice also comprising human TdT transgenes described in Example 1 above using the same sequencing method as described in Example 2 and the primers listed in **Table 5,** increased sequence diversity (about 2 fold) was observed at the mouse lambda locus of transgenic mice **(FIG. 9).** In addition, we observed increased rate of mouse immunoglobulin lambda non-template additions **(FIG. 10).** CDR3 lengths of the lambda chains in the TdT transgenic mice are depicted in **FIG. 11.** Finally, no difference in the mouse V lambda usage was observed between the various tested animals **(FIG. 12).**

Table 5: Primers used in library preparation for IgL-C1 repertoire sequencing

RT primers	**IgL (SEQ ID NO:24)**	5' - CACCAGTGTGGCCTTGTTAGTCTC- 3'
1st round PCR primers	**IgL Constant (SEQ ID NO:25)**	5′ - ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGGTGGAAACAGGGTGACTGATG - 3′
	PE2-PIIA (SEQ ID NO:21)	5′ - GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGCAGTGGTATCAACGCAGAGT - 3′
2nd round PCR Primers	**Forward (SEQ ID NO:22)**	5′ - AATGATACGGCGACCACCGAGATCTACACXXXXXXACACTCTTTCCCTACACGACGCTCTTCCGATCT - 3′
	Reverse (SEQ ID NO:23)	5′ - CAAGCAGAAGACGGCATACGAGATXXXXXXGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT- 3′

[0238] Additionally, to the VELOCIMMUNE® mice that comprise unrearranged human heavy and light chain variable gene segments, including those comprising functional ectopic mouse Adam6 gene as described above *(e.g.,* U.S. Patent Nos. 8,878,001; 9,078,418; 9,125,386), or mice comprising only unrearranged human heavy chain variable gene segments or unrearranged human light chain variable gene segments, other animals can be generated that contain a human TdTS. Some such animals include those comprising a human lambda variable region either on endogenous mouse lambda or kappa locus (US Patent Nos. 9,035,128; 9,066,502; 9,163,092; 9,120,662; 9,029,628; 9,006,511; 9,012,717), a human kappa variable region at the endogenous heavy chain locus *(e.g.,* U.S. Patent Application Publication No. 2012/0096572), a humanized TCR alpha and beta loci (*e.g.,* U.S. Patent No. 9,113,616) and various permutations thereof, dual light chain mice and permutations thereof (US Patent Application Publication No. 2013/0198880), universal light chain mice and permutations thereof *(e.g.,* US Patent Application Publication Nos. 2011/0195454; 2013/018582), universal heavy chain mice and permutations thereof (*e.g.,* US Patent No. 9,204,624), mice comprising histidine substitutions in their germline genome *(e.g.,* U.S. Patent Nos. 9,334,334 and 9,301,510, US Patent Application Publication Nos. 2013/0247236, 2014/0013456), chimeric antigen receptor mice *(e.g.,* US Patent Application Publication No. 2016/0081314), mice lacking a CH1 domain (*e.g.*, US Patent No. 8,754,287 and US Patent Application Publication No. 2015/0289489). Any such animals where one desires to increase junctional diversity either at the light and/or heavy chain (*e.g.*, human light and/or heavy chain) may be generated by introducing into ES cells comprising such modifications either a transgene or a targeted insertion of human TdTS described herein. In case of mice generated from ES cells comprising randomly integrated TdTS transgene (and in cases where IgK locus has not been modified, e.g., humanized TCR loci mice), they can be also generated by breeding with mice comprising various above-mentioned modifications. Successful incorporation of TdTS allele into such animals is determined as described herein above, and effect of human TdTS expression on generation of junctional diversity at various loci is determined as described herein above. The effect on non-modified rearranging loci, *e.g.,* endogenous mouse immunoglobulin and T cell loci, is also studied.

[0239] One such example, where the effect of TdTs introduction on junctional diversity of Dual Light Chain mice was studied, is presented in Examples 7-10 below.

Example 7. Human Immunoglobulin Kappa Junctional Diversity in Dual Light Chain (DLC) Mice Comprising Human TdTS

[0240] Mice comprising a dual light chain locus and human TdTS were generated by breeding VELOCIMMUNE® mice comprising a functional mouse Adam6 gene (see U.S. Patent Nos. 8,642,835 and 8,697,940) and exogenous human TdTS with mice comprising the dual light chain locus (see U.S. Patent Application Publication Number US 2013/0198880).

[0241] To assess immunoglobulin repertoire sequence diversity in DLC human TdTS mouse models that have a limited Igκ loci containing only two unrearranged V_K gene segments: IGVK3-20 and IGVK1-39, and five unrearranged IGJK gene segments (see U.S. Patent Application Publication Number US2013/0198880), Igκ sequences were amplified by 5' RACE from spleens of various mice with mIgK constant primer and sequenced using Illumina MiSeq. In most experiments, several mice heterozygous for Rag TdT Tg and homozygous for the DLC locus (Rag TdT tg (HET) DLC) and two mice homozygous for Rag TdT Tg and homozygous for the DLC locus (Rag TdT tg (HO) DLC) were used; data for Rag TdT tg (HET) DLC is depicted as mean of all mice tested, while the two Rag TdT tg (HO) DLC mice are shown individually.

[0242] Specifically, splenic B cells were positively enriched from total splenocytes by magnetic cell sorting using anti-CD19 (mouse) magnetic beads and MACS® columns (Miltenyi Biotech). Total RNA was isolated from the purified splenic B cells using an RNeasy Plus RNA isolation kit (Qiagen) according to manufacturer's instructions. Reverse transcription was performed to generate cDNA containing Igκ constant region sequence, using a SMARTer™ RACE cDNA Amplification Kit (Clontech) and an Igκ specific primer **(Table 4).** During this process, a DNA sequence, which is reverse compliment to 3' of primer PE2-PIIA, was attached to the 3' end of the newly synthesized cDNAs. Purified Igκ specific cDNAs were then amplified by the 1st round PCR using the PE2-PIIA primer and an Igκ constant specific primer listed in **Table 4.** PCR products between 450-700bp were isolated using Pippin Prep (SAGE Science). These products were further amplified by a 2nd round PCR using primers listed in **Table 4** ("XXXXXX" represents a 6bp index sequences to enable multiplexing samples for sequencing). PCR products between 400bp-700bp were isolated, purified, and quantified by qPCR using a KAPA Library Quantification Kit (KAPA Biosystems) before loading onto a Miseq sequencer (Illumina) for sequencing using Miseq Reagent Kits v3 (600 cycles).

[0243] For bioinformatics analysis, raw Illumina sequences were de-muliplexed and filtered based on quality, length and perfect match to kappa constant region primer. Overlapping paired-end reads were merged and analyzed using custom in-house pipeline. The pipeline used local installation of IgBLAST (NCBI, v2.2.25+) to align rearranged light chain sequences to human germline V and J gene database. Rearrangements were considered productive if no stop codons were detected and VJ junction was in-frame with J segment. Otherwise rearrangements were considered nonproductive and excluded from analysis.

[0244] CDR3 sequences were extracted using International Immunogenetics Information System (IMGT) boundaries. Junctional region between annotated V and J segments was classified as P and N nucleotides (non-template additions). Region with N/P additions was extracted from each sequence and its length calculated. Diversity of antibody repertoire was calculated by analyzing unique clonotypes. Sequence diversity was defined as a number of unique CDR3 sequences in randomly chosen 10,000 reads.

[0245] **FIG. 13** shows that over a 2-fold increase in the number of unique CDR3 amino acid sequences was detected in DLC human TdTS mouse models compared with DLC mice that did not comprise introduced human TdTS.

Example 8. Increase in Non-Germline Additions in DLC Mice Comprising Human TdTS

[0246] Percentage of non-germline nucleotide additions in CDR3 (which consists of parts of both Vκ and Jκ gene segments) in immunoglobulin sequences of DLC TdT mice was also determined from Next Generation Sequencing described in Example 7 above.

[0247] As depicted in **FIG. 14,** about half of humanized kappa light chains in B cells were shown to have non-germline additions in DLC humanized TdTS mice (both HET and HO for TdT) as compared to about 10% in DLC control mice (DLC mice with no introduced human TdT).

Example 9. Human Light Chain CDR3 Lengths in Immunoglobulins Obtained from DLC Mice Comprising Human TdTS

[0248] CDR3 sequences were extracted using International Immunogenetics Information System (IMGT) boundaries. Non-template nucleotides were determined from known light chain V and J sequences.

[0249] As depicted in **FIG. 15,** increased non-template additions observed in the DLC humanized TdTS mice (both HET and HO) described led to increase in kappa light chain CDR3 length compared to control (DLC mice with no introduced human TdT).

Example 10. Human Light Chain Vκ and Jκ Gene Segment Usage in DLC Mice Comprising Human TdTS

[0250] As depicted in **FIG. 16,** introduction of human TdTS in DLC mice did not significantly alter the usage of either Vκ gene segments or Jκ gene segments compared to DLC control mice (DLC mice with no introduced human TdT).

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PATENTKRAV

1. Fremgangsmåde til fremstilling af et antistof, der er specifikt for et antigen, hvor fremgangsmåden omfatter eksponering af en genetisk modificeret gnaver for antigenet, hvor den genetisk modificerede gnaver i sit genom omfatter:

en nukleinsyresekvens, der koder for en human, terminal deoxynucleotidyltransferase (TdT) operativt bundet til et transskriptionsstyringselement, der driver ekspression af nukleinsyresekvensen, som koder for den humane TdT i præ-B-celler; og

et variabelt område af det humane immunglobulin, der omfatter ikke-omarrangerede gensegmenter af det humane immunglobulins variable område, hvor det variable område af det humane immunglobulin er operativt bundet til et konstant område af immunglobulinet.

2. Fremgangsmåde ifølge krav 1, hvor fremgangsmåden endvidere omfatter:

(a) opnåelse af en nukleinsyresekvens, der koder for et variabelt domæne af det humane immunglobulin fra gnaverens antistof,

(b) operativ binding af nukleinsyresekvensen, der koder for det variable domæne af antistoffets humane immunglobulin, til en nukleinsyresekvens, der koder for et konstant domæne af et humant immunglobulin i en værtscelle; og

(c) dyrkning af værtscellen under forhold, således at værtscellen udtrykker et humant antistof, der omfatter det variable domæne af immunglobulinet og det konstante domæne af immunglobulinet.

3. Fremgangsmåde ifølge krav 2, hvor:

(a) det variable domæne af immunglobulinet er et variabelt domæne af immunglobulinets tungkæde, og det konstante domæne af immunglobulinet er et konstant domæne af immunglobulinets tungkæde; og/eller

(b) det variable domæne af immunglobulinet er et variabelt domæne af immunglobulinets letkæde, og det konstante domæne af immunglobulinet er et konstant domæne af immunglobulinets letkæde.

4. Fremgangsmåde til fremstilling af en nukleinsyre, der koder for et variabelt domæne af tungkæden af et humant immunglobulin, der er specifikt for et antigen, og/eller et variabelt domæne af letkæden af det humane immunglobulin, der er specifikt for et antigen, hvor fremgangsmåden omfatter:

(a) eksponering af en genetisk modificeret gnaver for antigenet, hvor den genetisk modificerede gnaver i sit genom omfatter:

en nukleinsyresekvens, der koder for en human, terminal deoxynucleotidyltransferase (TdT) operativt bundet til et transskriptionsstyringselement, der driver ekspression af nukleinsyresekvensen, der koder for den humane TdT i præ-B-celler; og

et variabelt område af det humane immunglobulin, der omfatter ikke-omarrangerede gensegmenter fra det humane immunglobulins variable område, hvor det variable område af det humane immunglobulin er operativt bundet til et konstant område af immunglobulinet; og

(b) opnåelse af en nukleinsyresekvens, der koder for et variabelt domæne af tungkæden af et humant immunglobulin, der er specifikt for antigenet, og/eller et variabelt domæne af letkæden af det humane immunglobulin, der er specifikt for antigenet fra gnaveren.

5. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 4, hvor transskriptionsstyringselementet:

(i) driver ekspression af nukleinsyresekvensen, der koder for den humane TdT i pro-B-celler og præ-B-celler; og/eller

(ii) vælges fra gruppen bestående af et RAG1-transskriptionsstyringselement, et RAG2-transskriptionsstyringselement, et transskriptionsstyringselement af immunglobulinets tungkæde, et transskriptionsstyringselement af immunglobulinets κ-letkæde og/eller et transskriptionsstyringselement af immunglobulinets λ-letkæde.

6. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 5, hvor den humane TdT ikke udtrykkes konstitutivt.

7. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 6, hvor nukleinsyresekvensen, der koder for den humane TdT, er placeret ved et immunglobulin κ-letkædelocus, et immunglobulin λ-letkædelocus, et immunglobulin tungkædelocus, et RAG1-locus eller et RAG2-locus.

8. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 7, hvor det variable område af det humane immunglobulin er et variabelt område af det humane immunglobulins tungkæde omfattende ikke-omarrangerede, humane immunglobulin-tungkæde V-, D- og J-gensegmenter, og genet fra det konstante immunglobulinområde er et konstant område af immunglobulinets tungkæde.

9. Fremgangsmåde ifølge krav 8, hvor:

(i) genet fra det konstante område af immunglobulinets tungkæde er af endogen artsoprindelse; og/eller (ii) det variable område af tungkæden af det humane immunglobulin og genet fra det konstante område af immunglobulinets tungkæde er placeret ved et endogent immunglobulintungkædelocus.

10. Fremgangsmåde ifølge krav 8 eller 9, og som endvidere i sit genom omfatter et variabelt område af det humane immunglobulins letkæde omfattende ikke-omarrangerede gensegmenter af det variable område af den humane letkæde operativt bundet til et konstant område af immunglobulinets letkæde, eventuelt hvor det variable område af immunglobulinets letkæde operativt bundet til det konstante område af immunglobulinets letkæde er placeret ved et endogent locus for immunglobulinets letkæde.

11. Fremgangsmåde ifølge krav 10, hvor

(i) det variable område af letkæden af det humane immunglobulin omfatter gensegmenter fra det variable område af den humane κ-kæde;

(ii) det konstante område af immunglobulinets letkæde er et konstant område af immunglobulin κ;

(iii) det konstante område af immunglobulinets letkæde er et konstant område af immunglobulin κ af endogen artsoprindelse; og/eller

(iv) det variable område af letkæden af det humane immunglobulin og det konstante område af immunglobulinets letkæde er placeret ved et endogent locus for immunglobulinets letkæde κ.

12. Fremgangsmåde ifølge krav 10, hvor:

(i) det variable område af letkæden af det humane immunglobulin omfatter gensegmenter af det variable område af den humane λ-kæde;

(ii) det konstante område af immunglobulinets letkæde er et konstant immunglobulin λ-område;

(iii) det konstante område af immunglobulinets letkæde er et konstant immunglobulin λ-område af endogen artsoprindelse; og/eller

(iv) det variable område af letkæden af det humane immunglobulin og det konstante område af immunglobulinets letkæde er placeret ved et endogent locus for immunglobulinets letkæde λ.

13. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 12, hvor de ikke-omarrangerede gensegmenter fra det humane immunglobulins variable område undergår omarrangering under B-celleudvikling for at generere omarrangerede variable områder af det humane immunglobulin i B-celler fra det ikke-humane dyr, eventuelt hvor:

(i) mindst 10 % af generne fra det omarrangerede variable område omfatter ikke-template tilføjelser; og/eller

(ii) mindst 10 % af V-J-forbindelserne af immunglobulinets letkæde hos dyret omfatter ikke-template tilføjelser.

14. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 13, hvor gnaveren er en rotte eller en mus, eventuelt hvor gnaveren er en mus.

15. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor den humane TdT er den korte isoform af TdT (TdTS).

DRAWINGS

Drawing

Figure 1

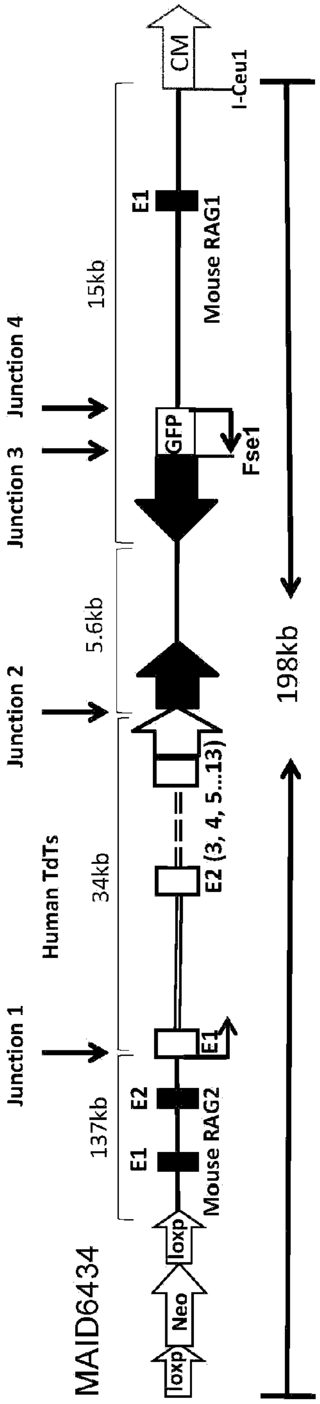


Figure 2

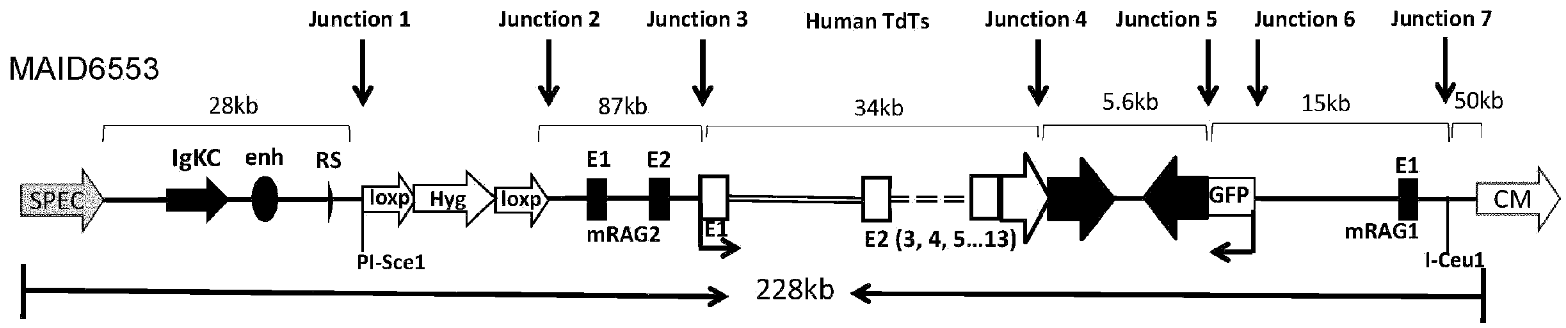
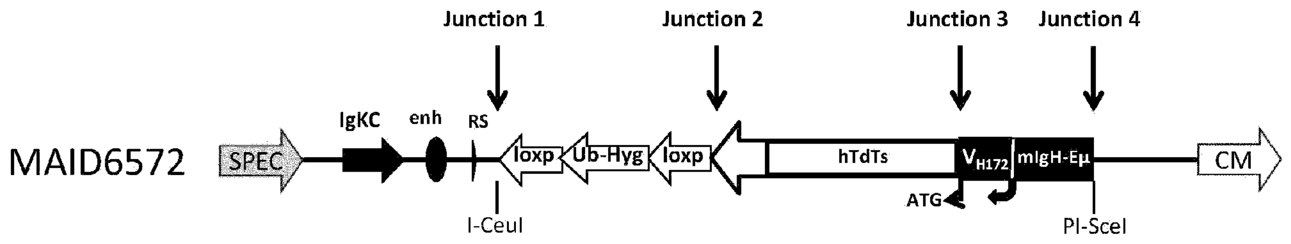

MAID6553
Junction 1
Junction 2
Junction 3
Human TdTs
Junction 4
Junction 5
Junction 6
Junction 7
28kb
87kb
34kb
5.6kb
15kb
50kb
SPEC
IgKC
enh
RS
loxp
Hyg
loxp
PI-Sce1
E1
E2
mRAG2
E1
E2 (3, 4, 5...13)
GFP
E1
mRAG1
I-Ceu1
CM
228kb

Figure 3


MAID6572
Junction 1
Junction 2
Junction 3
Junction 4
SPEC
IgKC
enh
RS
loxp
Ub-Hyg
loxp
hTdTs
V_{H172}
mIgH-Eμ
CM
I-CeuI
ATG
PI-SceI

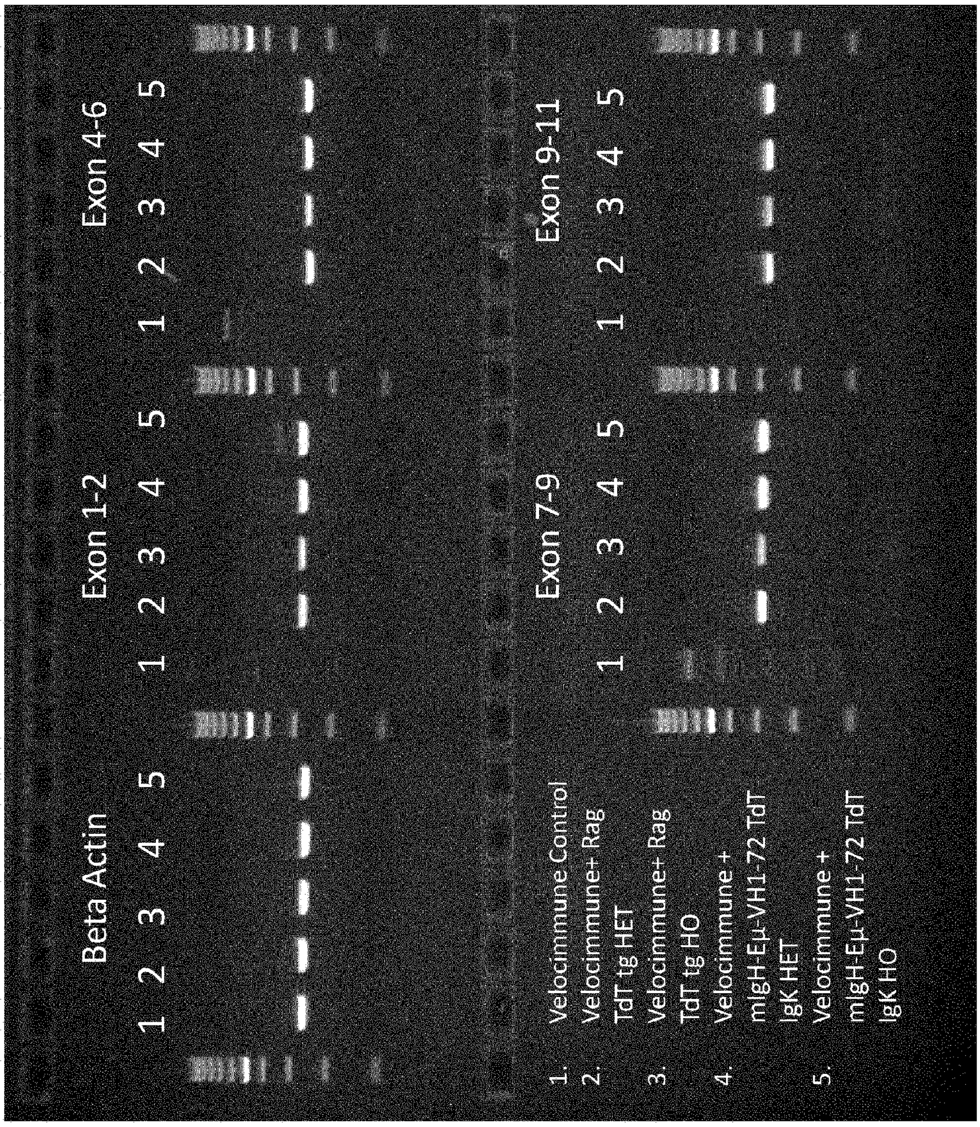

Beta Actin
1 2 3 4 5
Exon 1-2
1 2 3 4 5
Exon 4-6
1 2 3 4 5
Exon 7-9
1 2 3 4 5
Exon 9-11
1 2 3 4 5
1. Velocimmune Control
2. Velocimmune+ Rag TdT tg HET
3. Velocimmune+ Rag TdT tg HO
4. Velocimmune + mIgH-Eμ-VH1-72 TdT IgK HET
5. Velocimmune + mIgH-Eμ-VH1-72 TdT IgK HO

Figure 4

Figure 5

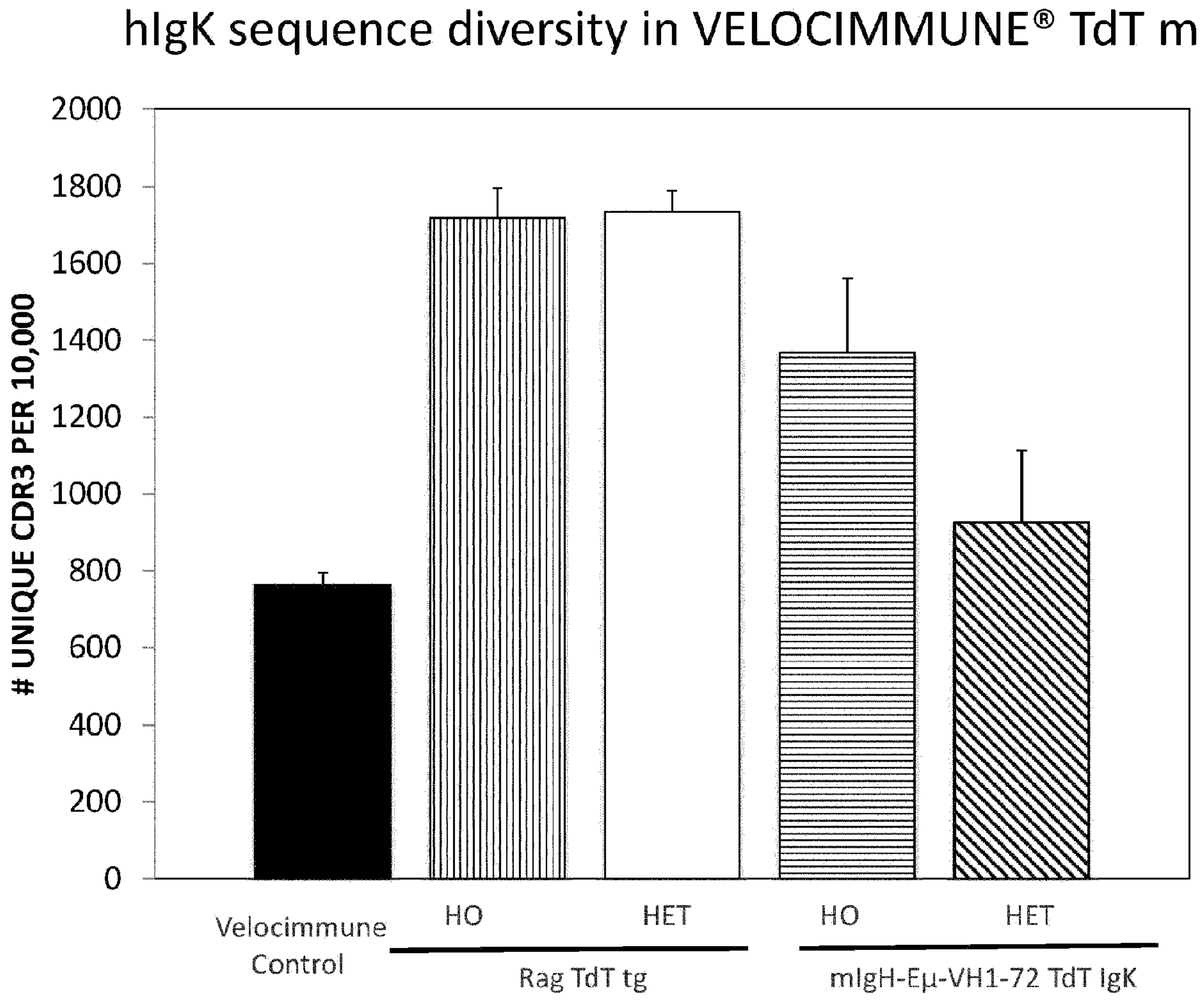

hIgK sequence diversity in VELOCIMMUNE® TdT mice
UNIQUE CDR3 PER 10,000
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Velocimmune Control
HO
HET
Rag TdT tg
HO
HET
mIgH-Eμ-VH1-72 TdT IgK

Figure 6

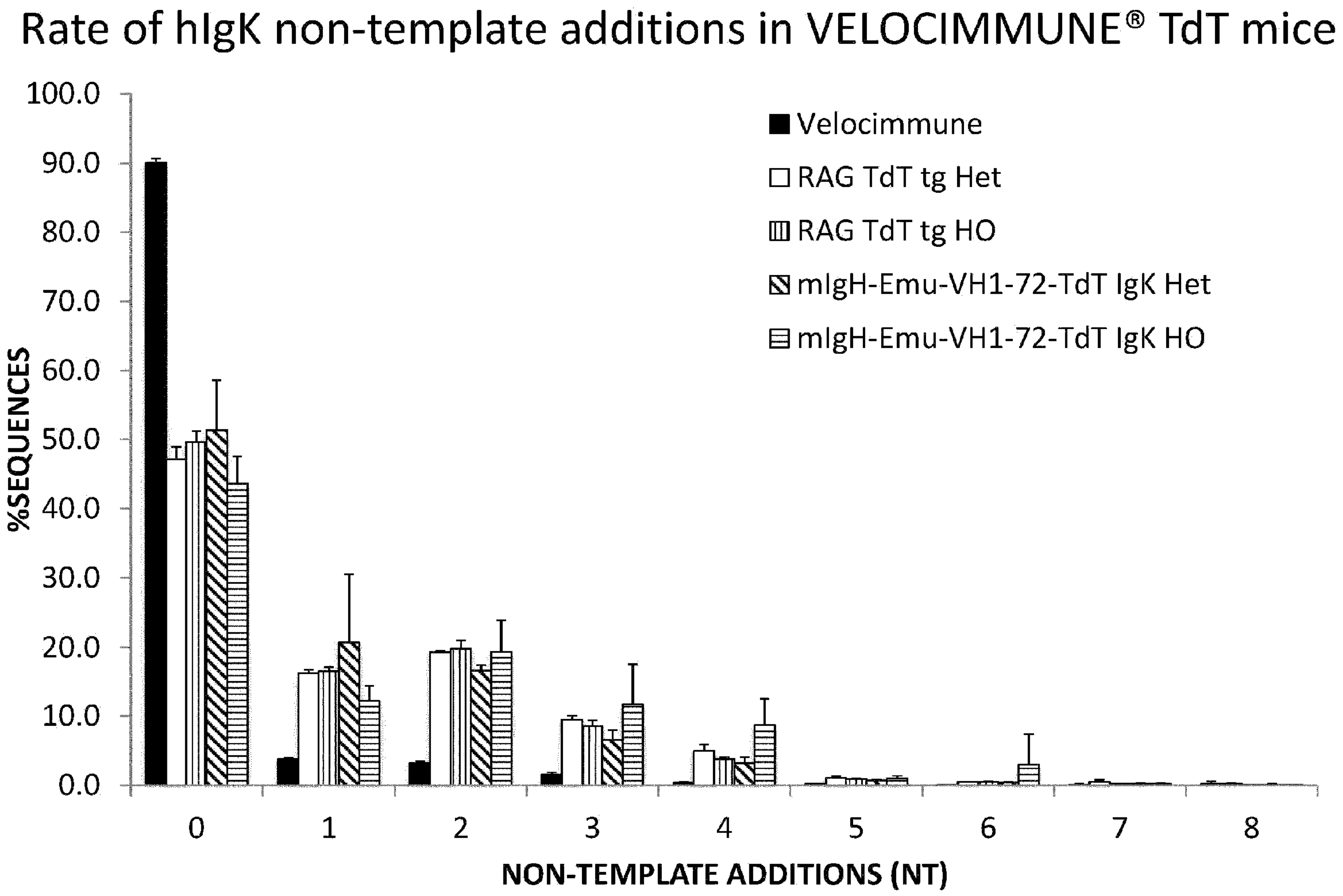
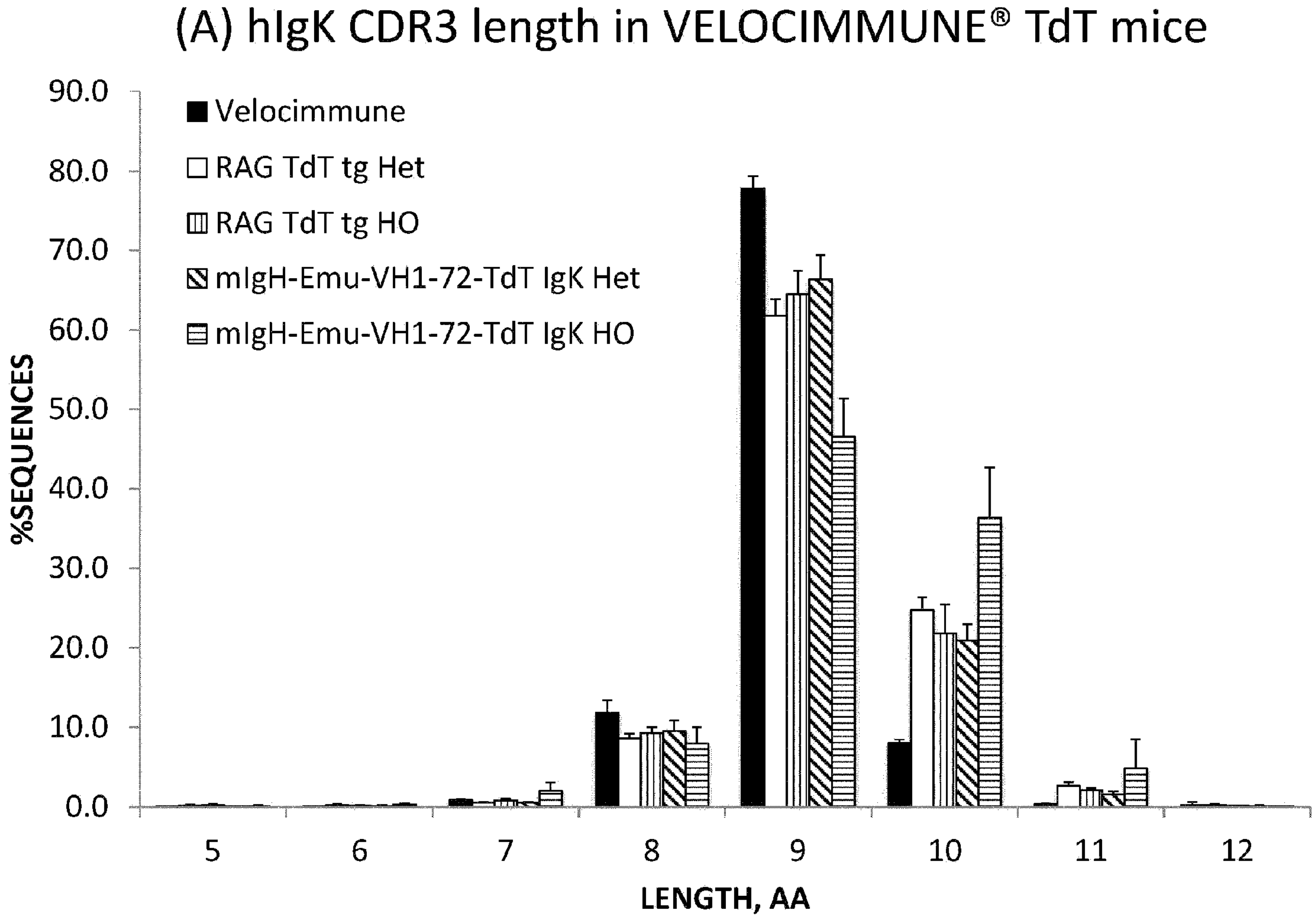

Rate of hIgK non-template additions in VELOCIMMUNE® TdT mice
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
%SEQUENCES
■ Velocimmune
☐ RAG TdT tg Het
▥ RAG TdT tg HO
▧ mIgH-Emu-VH1-72-TdT IgK Het
▤ mIgH-Emu-VH1-72-TdT IgK HO
0
1
2
3
4
5
6
7
8
NON-TEMPLATE ADDITIONS (NT)

Figure 7



(A) hIgK CDR3 length in VELOCIMMUNE® TdT mice

Figure 7 (Continued)

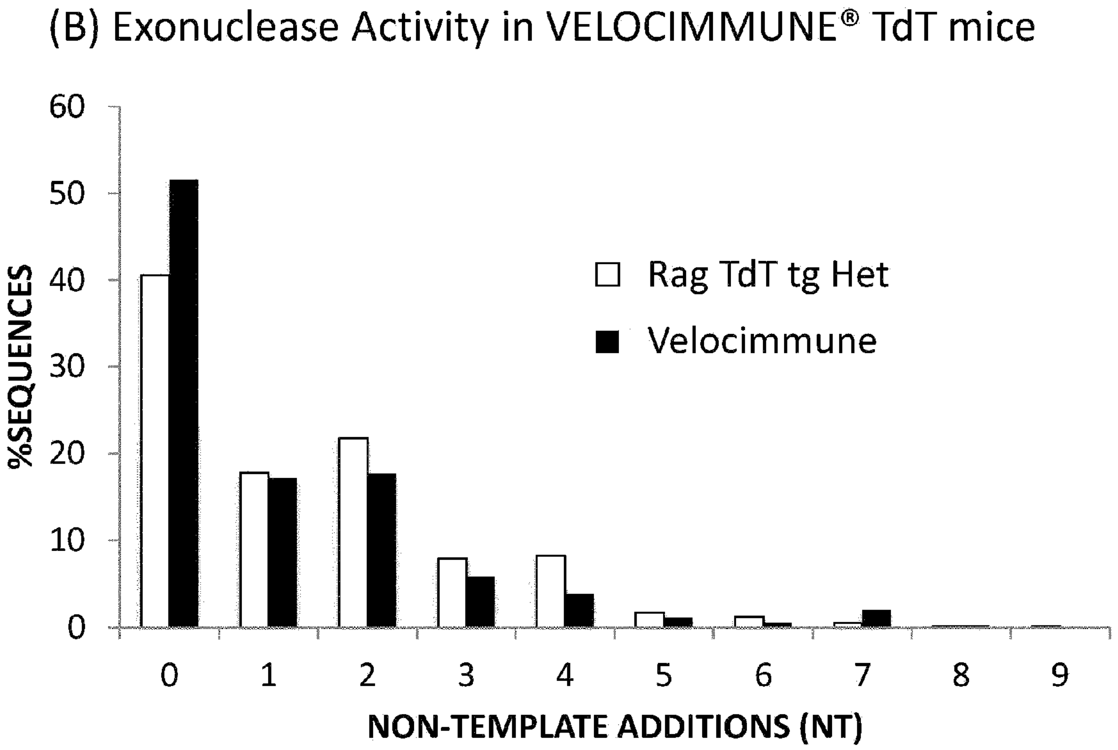


Figure 8

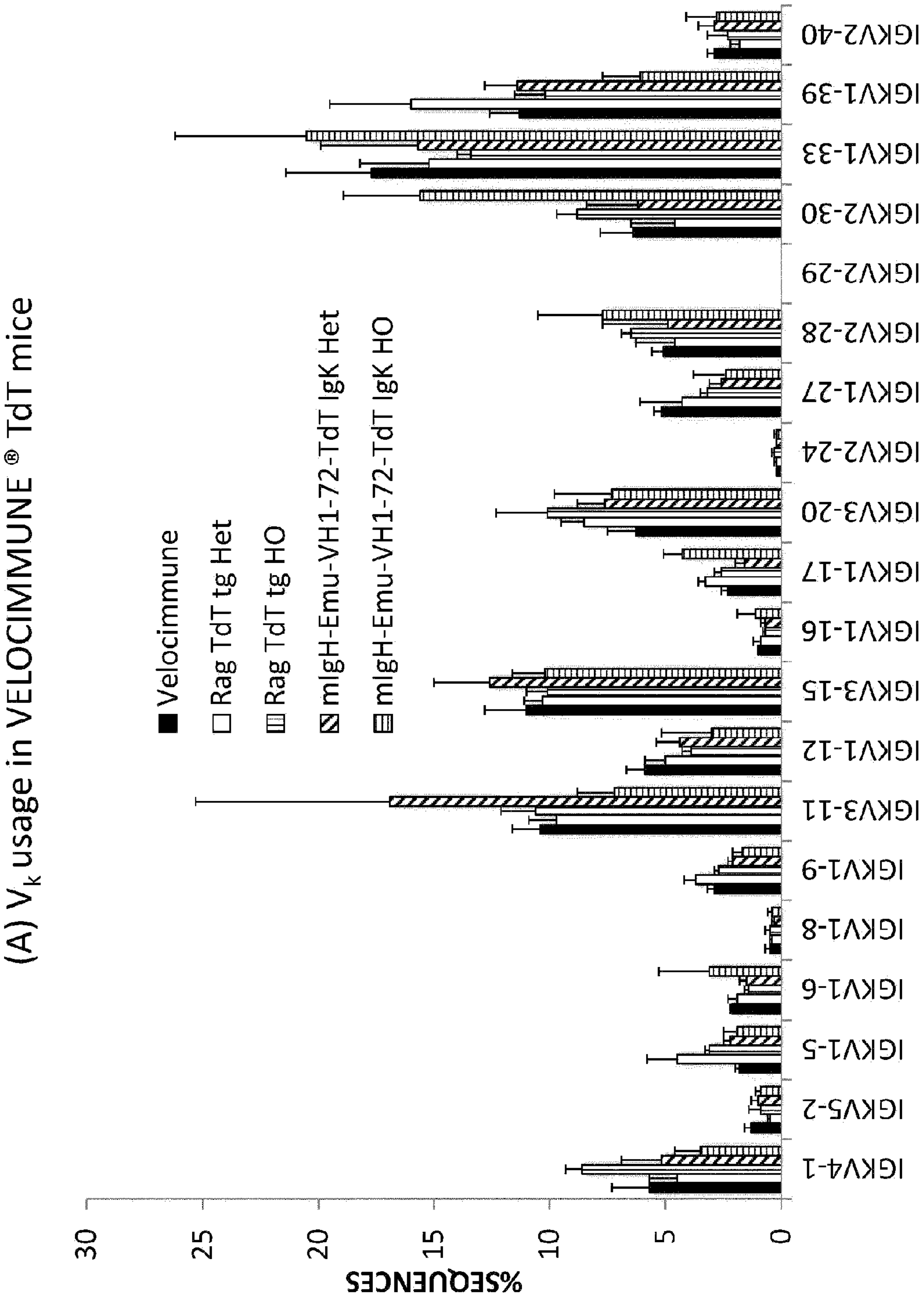

(A) V_k usage in VELOCIMMUNE ® TdT mice
■ Velocimmune
□ Rag TdT tg Het
▤ Rag TdT tg HO
▨ mIgH-Emu-VH1-72-TdT IgK Het
▤ mIgH-Emu-VH1-72-TdT IgK HO
%SEQUENCES
30
25
20
15
10
5
0
IGKV4-1
IGKV5-2
IGKV1-5
IGKV1-6
IGKV1-8
IGKV1-9
IGKV3-11
IGKV1-12
IGKV3-15
IGKV1-16
IGKV1-17
IGKV3-20
IGKV2-24
IGKV1-27
IGKV2-28
IGKV2-29
IGKV2-30
IGKV1-33
IGKV1-39
IGKV2-40

Figure 8 (Continued)

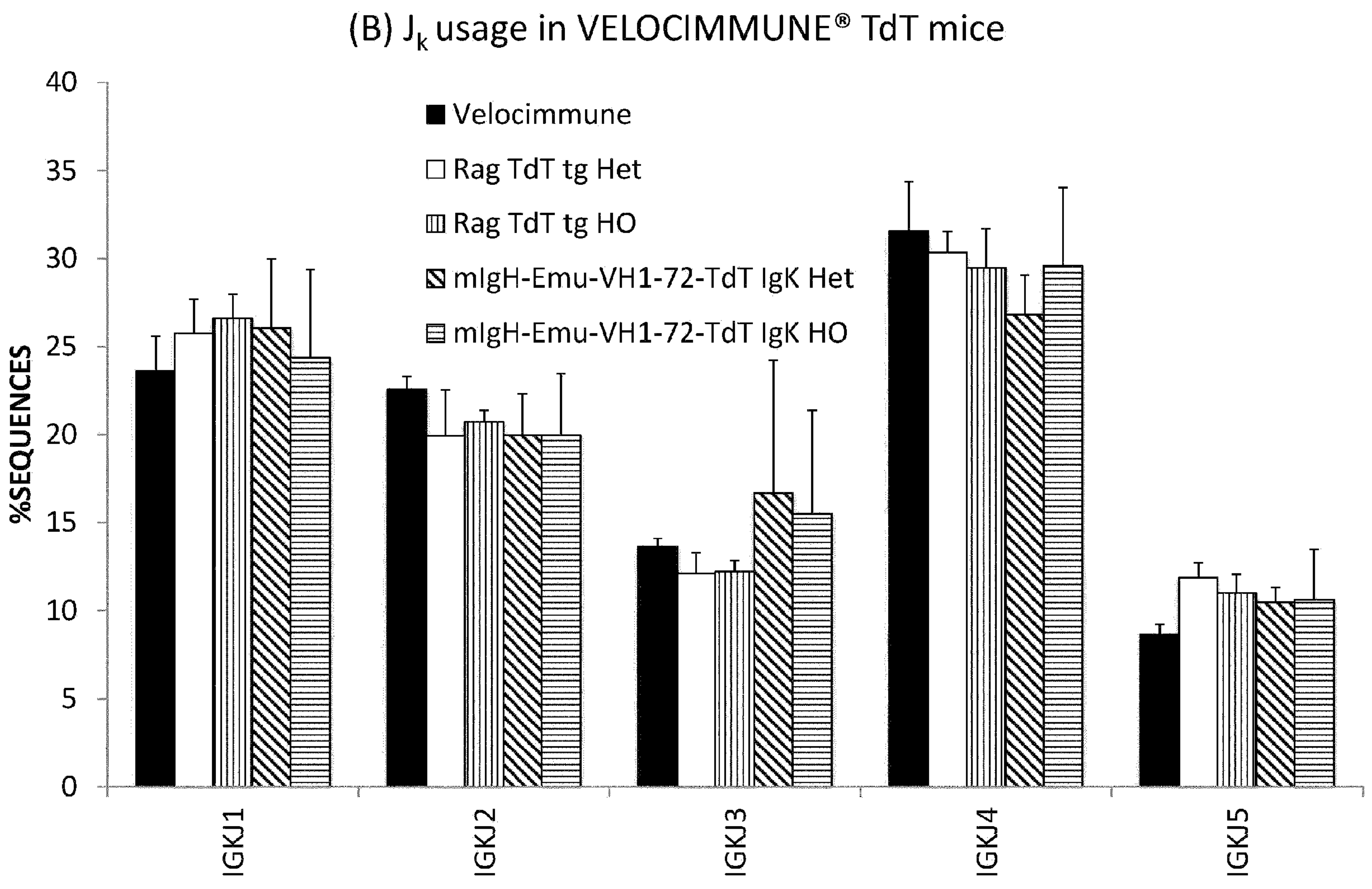


Figure 9

mIgλ sequence diversity in VELOCIMMUNE® TdT mice

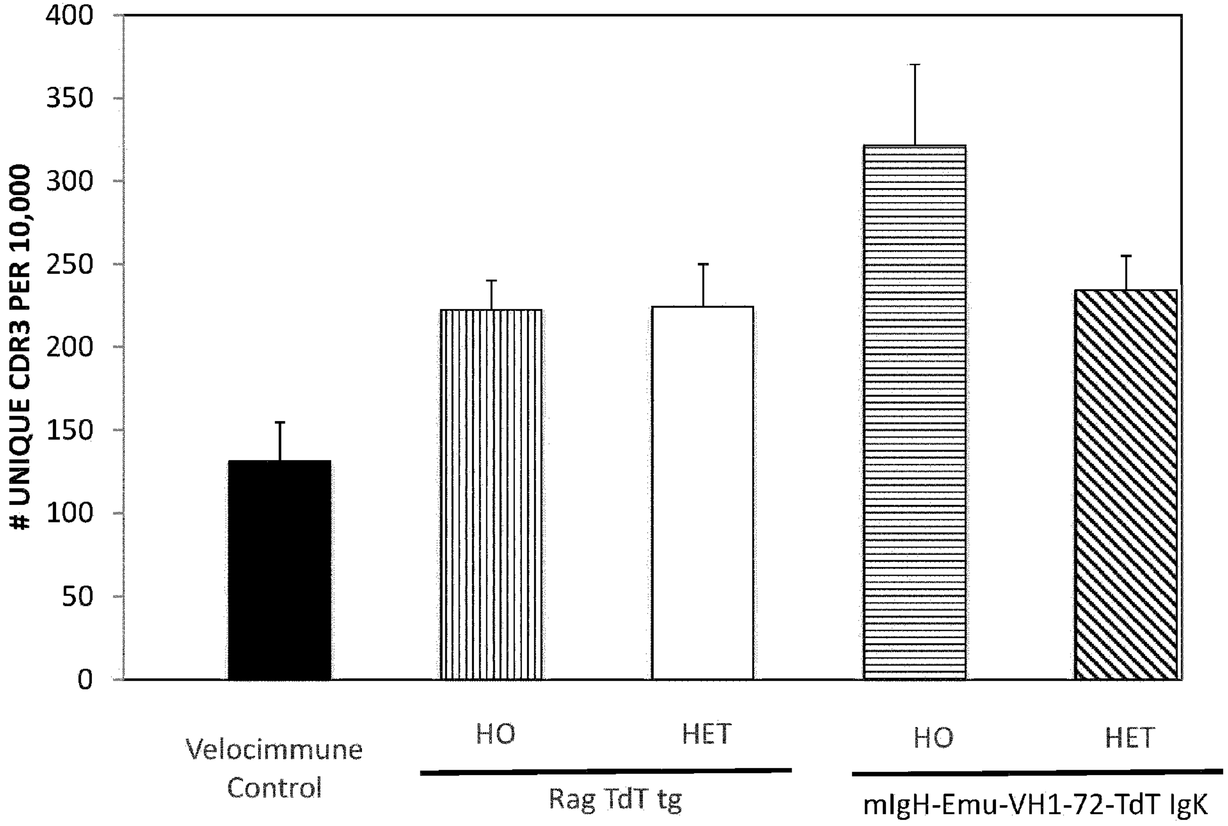


Figure 10

Rate of mIgλ non-template additions in VELOCIMMUNE® TdT mice

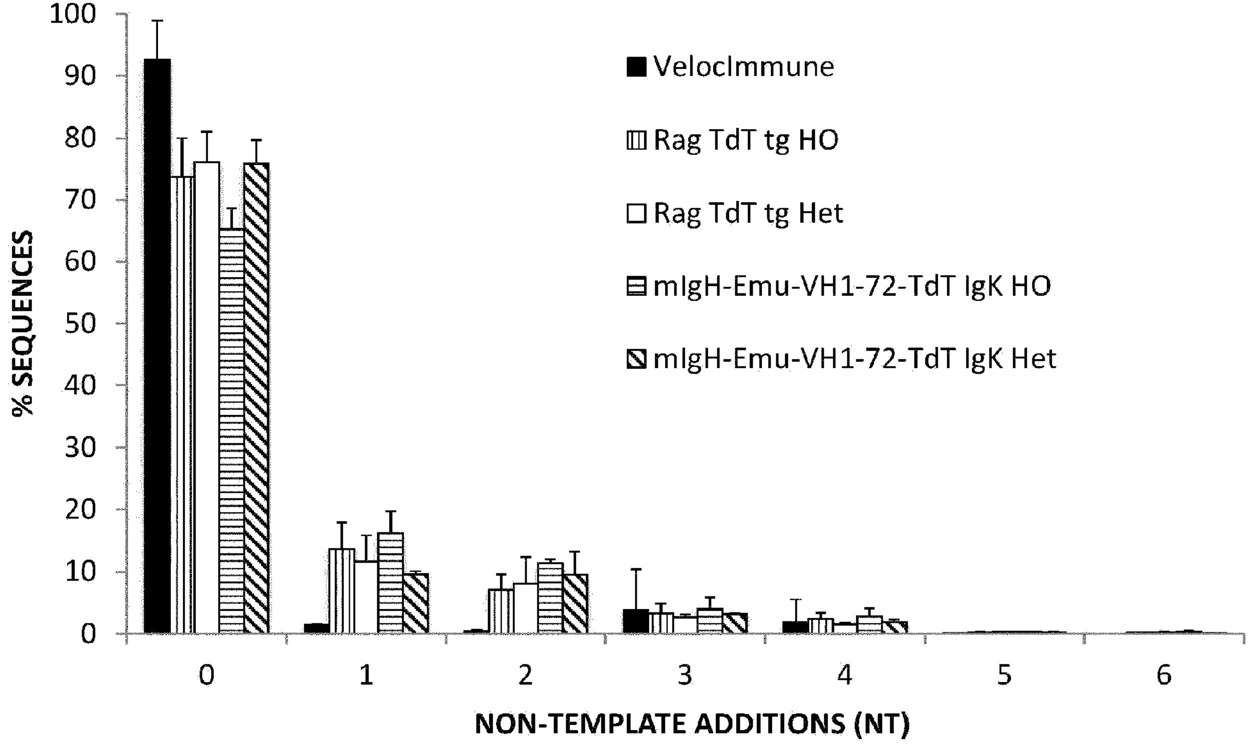


Figure 11

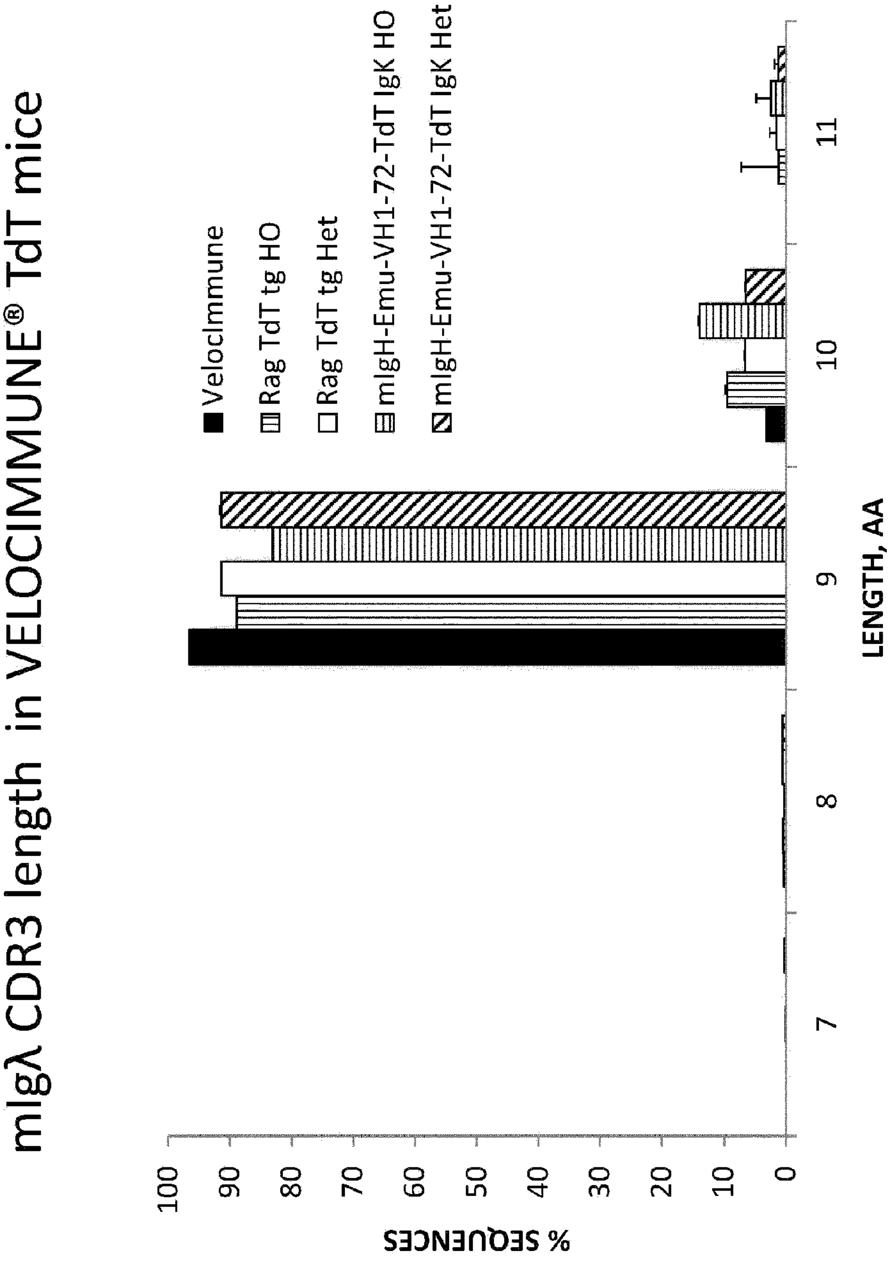

mIgλ CDR3 length in VELOCIMMUNE® TdT mice
■ VelocImmune
▥ Rag TdT tg HO
☐ Rag TdT tg Het
▤ mIgH-Emu-VH1-72-TdT IgK HO
▨ mIgH-Emu-VH1-72-TdT IgK Het
% SEQUENCES
100
90
80
70
60
50
40
30
20
10
0
7
8
9
10
11
LENGTH, AA

Figure 12

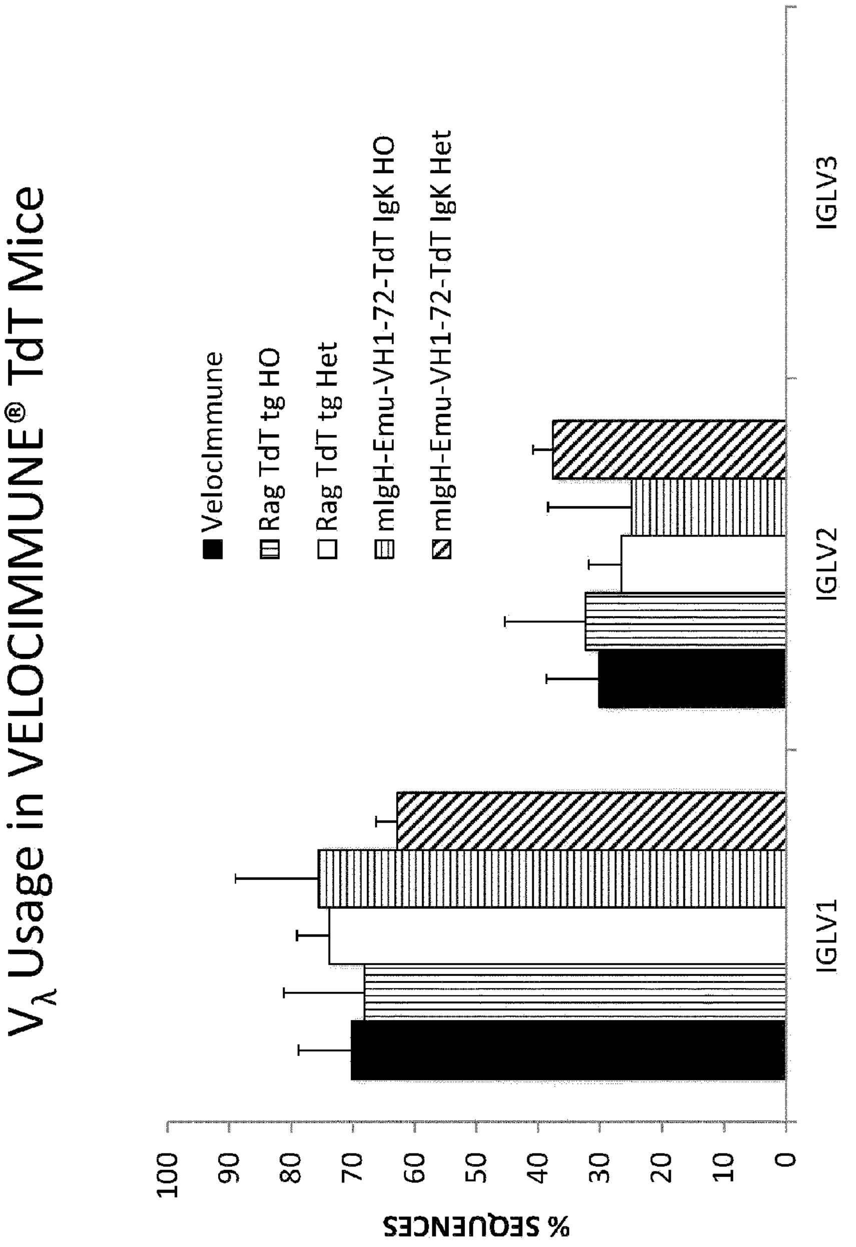
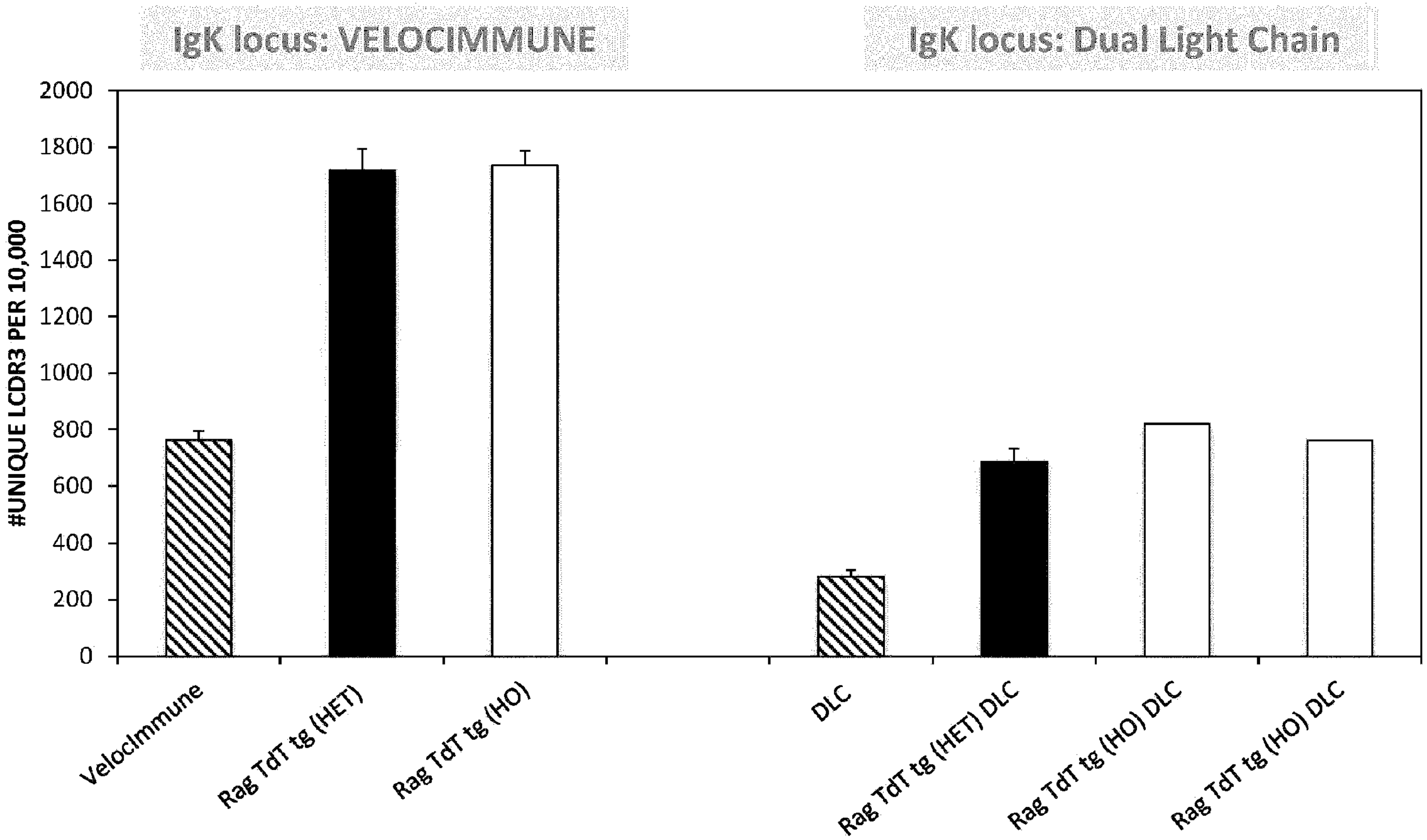

V_λ Usage in VELOCIMMUNE® TdT Mice
■ VelocImmune
▥ Rag TdT tg HO
☐ Rag TdT tg Het
▤ mIgH-Emu-VH1-72-TdT IgK HO
▨ mIgH-Emu-VH1-72-TdT IgK Het
% SEQUENCES
100
90
80
70
60
50
40
30
20
10
0
IGLV1
IGLV2
IGLV3

Figure 13



hIgK sequence diversity in Dual Light Chain TdT mice

Figure 14

Rate of mIgK non-template additions in Dual Light Chain TdT mice

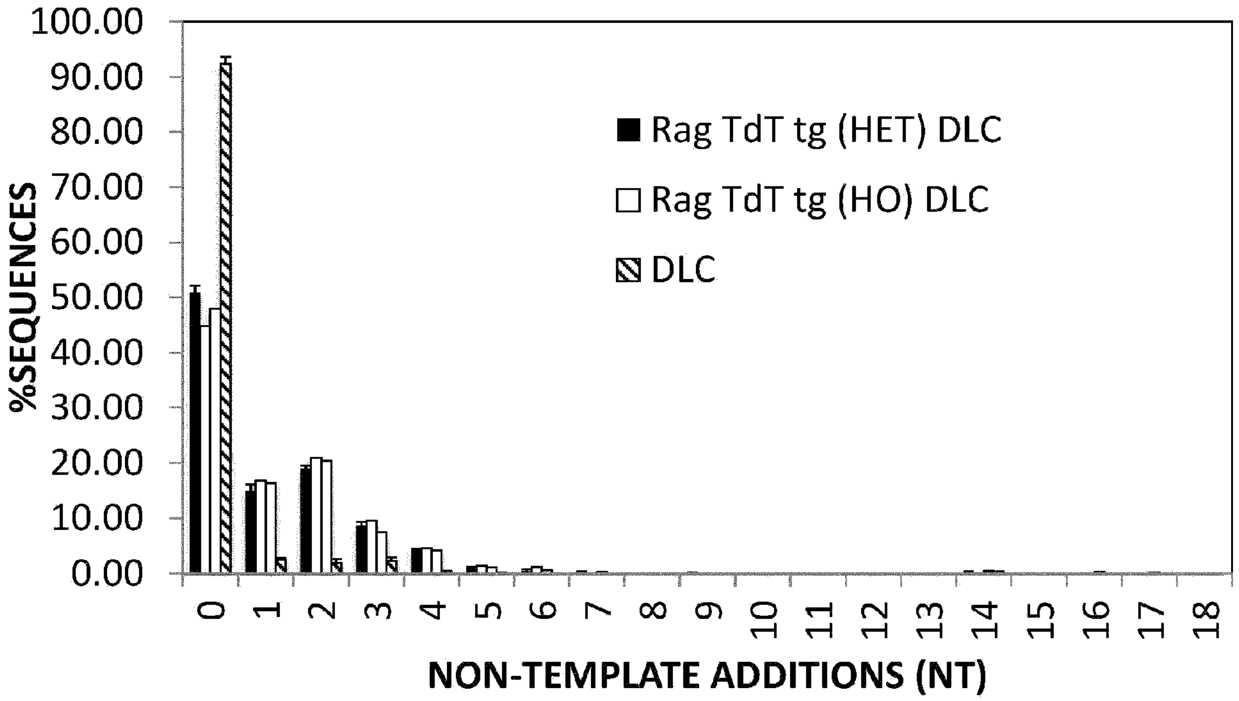


Figure 15

mIgK CDR3 length in Dual Light Chain TdT mice

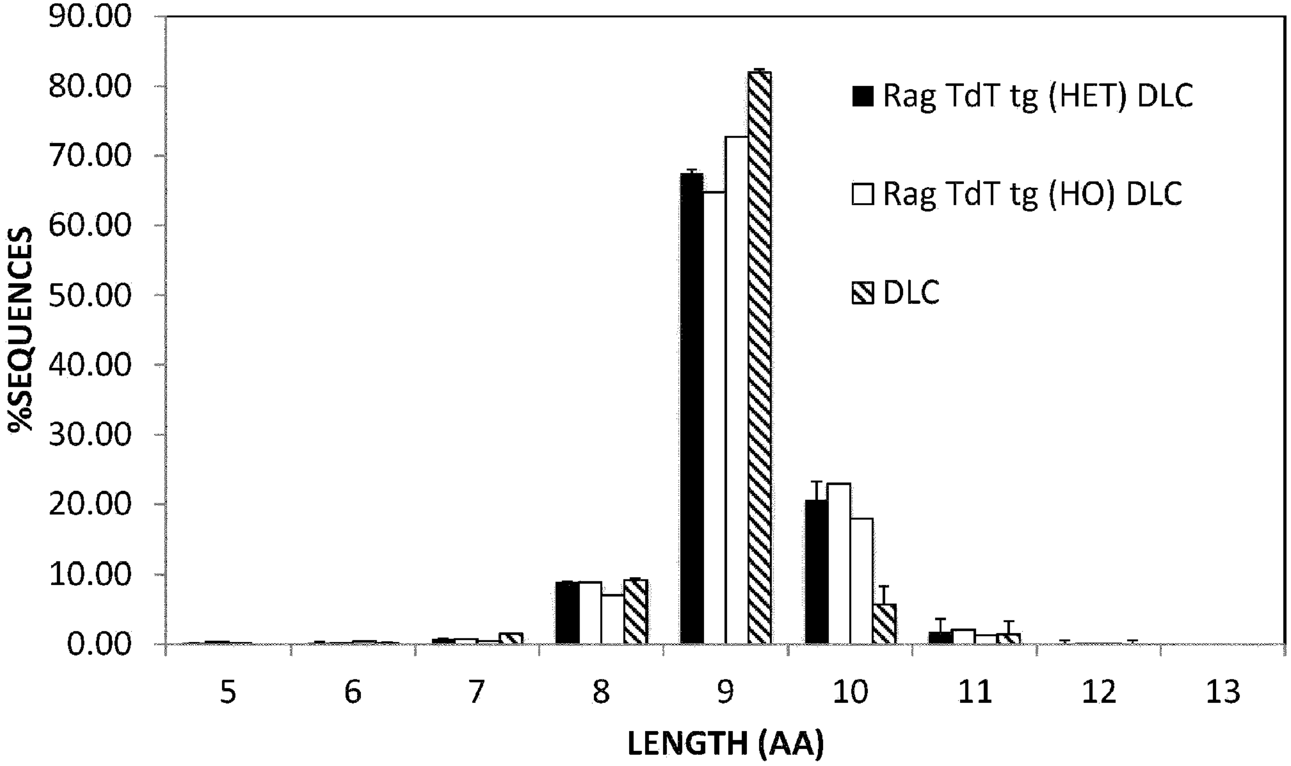
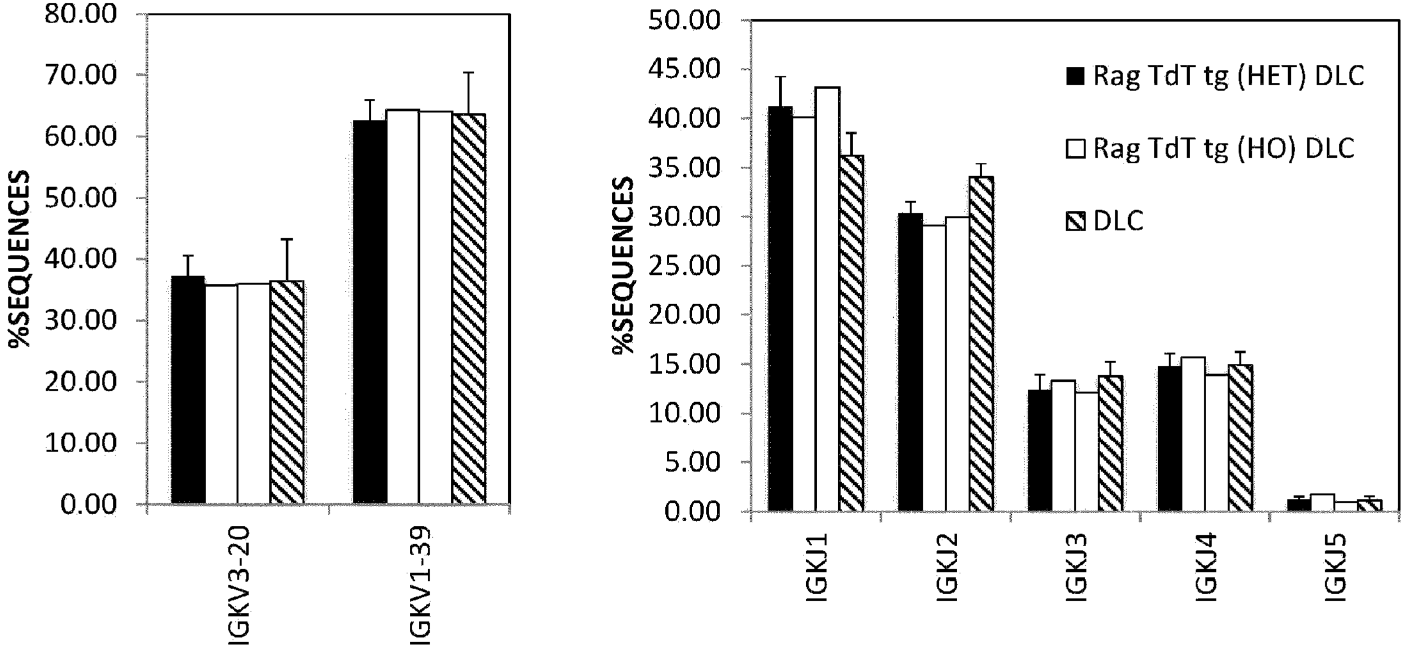


Figure 16

VK and JK Usage in Dual Light Chain TdT Mice



SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.