CLAIMS

1. A nucleotide molecule comprising an engineered immunoglobulin heavy chain diversity (D_H) ~~gene segment that comprises at least one D_H gene segment operably linked to a 23-mer recombination signal sequence (RSS).~~

~~2. The nucleotide molecule of claim 1, wherein the nucleotide sequence comprises an engineered D_H~~ region ("engineered D_H region") comprising~~:~~

(i) ~~the at least one~~an engineered D_H gene segment comprising a D_H gene segment ~~operably linked~~immediately adjacent to a 23-mer RSS ("engineered D_H gene segment") and (ii) an unrearranged D_H gene segment flanked on ~~one~~its 5' side by a first 12-mer RSS and on ~~the other~~its 3' side by ~~another12-mer RSS, and~~

~~wherein the (i) at least one D_H gene segment operably linked to~~ a ~~23~~second 12-mer RSS ~~and the (ii)~~ ("unrearranged D_H gene segment"),

wherein (i) the engineered D_H gene segment ~~flanked on one side by a 12-mer RSS and on the other side by another12-mer RSS~~and (ii) the unrearranged D_H gene segment are operably linked such that (i) the engineered D_H gene segment and (ii) the unrearranged D_H gene segment are able to join in a D_H-D_H recombination event according to the 12/23 rule,

wherein the nucleotide molecule is suitable for obtaining rodents having the engineered D_H region, and

optionally wherein the engineered D_H region comprises only human D_H gene segments.

~~3~~2. The nucleotide molecule of claim ~~2~~1, wherein the nucleotide molecule further comprises:

(a) at least one unrearranged immunoglobulin heavy chain variable (V_H) gene segment ("unrearranged V_H gene segment") that is upstream of and operably linked to the engineered D_H region~~,~~;

(b) at least one unrearranged immunoglobulin heavy chain joining (J_H) gene segment ("unrearranged J_H gene segment") that is downstream of and operably linked to the engineered D_H region~~,~~; or

(c) a combination of (a) and (b).

43. The nucleotide molecule of claim 32, wherein

(a) the at least one unrearranged V_H gene segment comprises an unrearranged human V_H6-1 gene segment, the at least one unrearranged J_H gene segment comprises an unrearranged human J_H6 gene segment, or a combination thereof;

(b) 5. The nucleotide molecule of claim 3 or claim 4, wherein the at least one unrearranged J_H gene segment comprises an unrearranged human J_H4 gene segment, an unrearranged human J_H5 gene segment, and an unrearranged human J_H6 gene segment;

(c) 6. The nucleotide molecule of any one of claims 3-5, wherein the at least one unrearranged J_H gene segment comprises an unrearranged human J_H1 gene segment, an unrearranged human J_H2 gene segment, an unrearranged human J_H3 gene segment, an unrearranged human J_H4 gene segment, an unrearranged human J_H5 gene segment, and an unrearranged human J_H6 gene segment, optionally wherein the unrearranged human J_H1 gene segment, the unrearranged human J_H2 gene segment, the unrearranged human J_H3 gene segment, the unrearranged human J_H4 gene segment, the unrearranged human J_H5 gene segment, and the unrearranged human J_H6 gene segmentssegment are in germline configuration; and/or

(d) 7. The nucleotide molecule of any one of claims 3-6, whereinthe at least one unrearranged V_H gene segment comprises the full repertoire of functional unrearranged human V_H gene segments spanning between and including thean unrearranged human V_H3-74 gene segment and an unrearranged human V_H1-6 gene segment ("full repertoire of functional unrearranged human V_H gene segments"), optionally wherein the full repertoire of functional unrearranged human V_H gene segments is in germline configuration.

84. The nucleotide molecule of any one of the preceding claims, wherein:

(a) the nucleotide molecule further comprisingcomprises one or more functional rodent Adam6 genes, optionally wherein the one or more functional rodent Adam6 genes is located between

a human V_H1-2 gene segment and a human V_H6-1 gene segment and/or replaces a human Adam6 gene~~.~~;

~~9. The nucleotide molecule of any one of~~ (b) the ~~preceding claims, wherein~~engineered D_H gene segment comprises the 23-mer RSS ~~is contiguous~~immediately adjacent to the 5'-end of the ~~at least one~~ D_H gene segment~~.~~;

~~10. The nucleotide molecule of any one of~~ (c) the ~~preceding claims, wherein~~engineered D_H gene segment comprises the 23-mer RSS ~~is contiguous~~immediately adjacent to the 3'-end of the ~~at least one~~ D_H gene segment~~.~~;

~~11. The nucleotide molecule of any one of~~ (d) the ~~preceding claims, wherein the at least one~~engineered D_H gene segment ~~operably linked to a 23-mer RSS~~ comprises:

(~~a~~i) a human D_H3-3 gene segment ~~operably linked~~immediately adjacent to ~~a~~the 23-mer RSS, optionally wherein the 23-mer RSS is ~~contiguous~~immediately adjacent to the 5'-end of the D_H3-3 gene segment~~,~~;

(~~b~~ii) a human D_H2-2 gene segment ~~operably linked~~immediately adjacent to ~~a~~the 23-mer RSS, optionally wherein the 23-mer RSS is ~~contiguous~~immediately adjacent to the 3'-end of the D_H2-2 gene segment~~,~~;

(~~c~~iii) a human D_H2-8 gene segment ~~operably linked~~immediately adjacent to ~~a~~the 23-mer RSS, optionally wherein the 23-mer RSS is ~~contiguous~~immediately adjacent to the 3'-end of the D_H2-8 gene segment~~,~~;

(~~d~~iv) a human ~~2~~D_H2-15 gene segment ~~operably linked~~immediately adjacent to ~~a~~the 23-mer RSS, optionally wherein the 23-mer RSS is ~~contiguous~~immediately adjacent to the 3'-end of the D_H2-15 gene segment~~,~~; or

(~~e~~v) any combination of (~~a) (d).~~i)-(iv);

(e) ~~12. The~~the nucleotide molecule ~~of any one of the preceding claims, comprising a~~comprises the nucleotide sequence ~~comprising the sequence~~ set forth as SEQ ID NO:52~~.~~

~~13. The nucleotide molecule of any one of claims 1-11, comprising a nucleotide sequence comprising the~~ or comprises the nucleotide sequence set forth as SEQ ID NO:61~~.~~;

(f) ~~14. The~~the nucleotide molecule ~~of any one of claims 1-13, comprising a nucleotide sequence comprising~~comprises the nucleotide sequence set forth as SEQ ID NO:70~~.~~;

~~15. The~~ (g) the nucleotide molecule ~~of any one of claims 1-14, comprising a nucleotide sequence comprising~~comprises the nucleotide sequence set forth as SEQ ID NO:71~~.~~;

~~16. The~~ (h) the nucleotide molecule ~~of any one of claims 1-15, comprising a nucleotide sequence comprising~~comprises the nucleotide sequence set forth as SEQ ID NO:72.

~~17. A~~5. The nucleotide molecule of any one of claims 1-4, comprising in operable linkage from 5' to 3':

(a) at least one ~~or more~~ unrearranged human V_H gene ~~segments~~segment;

(b) ~~an~~the engineered D_H region ~~comprising from 5' to 3'~~

~~(i) one or more unrearranged human D_H gene segments~~, wherein ~~each of the one or more of human D_H gene segments is flanked on its 5' and 3' ends by a 12-mer RSS, and~~

~~(ii) at least one~~the engineered D_H gene segment ~~operably linked to a 23-mer RSS comprising~~comprises a human D_H3-3 gene segment ~~operably linked at its 5'~~ immediately adjacent to the 23-mer RSS, wherein the 23-mer RSS is immediately adjacent to the 5' end ~~to a 23-mer RSS;~~of the human D_H3-3 gene segment, and

(c) at least one ~~or more~~ unrearranged human J_H gene ~~segments~~segment.

~~18. A~~6. The nucleotide molecule of any one of claims 1-4, comprising in operable linkage from 5' to 3':

(a) at least one ~~or more~~ unrearranged human V_H gene ~~segments;~~segment,

(b) ~~an~~the engineered D_H region, wherein the engineered D_H ~~region comprises from 5' to 3'~~

~~(i) at least one D_H~~ gene segment ~~operably linked to~~ comprises a ~~23-mer RSS comprising at least one~~ human D_H2 gene segment ~~operably linked at its 3' end~~immediately adjacent to ~~a~~the 23~~-~~-mer RSS, ~~optionally~~ wherein the ~~at least one human D_H2 gene segment operably linked at its~~23-mer RSS is immediately adjacent to the 3'end ~~to a 23-mer RSS comprises a~~of the human D_H2-~~2~~ gene segment ~~operably linked at its 3' end to a 23-mer RSS, a human D_H2-8 gene segment operably linked at its 3' end to a 23-mer RSS, a human D_H2-15 gene segment operably linked at its 3' end to a 23-mer RSS, or any combination thereof, and~~, optionally wherein:

~~(ii) one or a plurality of human D_H gene segments, wherein each of the one or plurality of human D_H gene segments is flanked on its 5' and 3' ends by a 12-mer RSS; and~~

(i) the engineered D_H gene segment comprises a human D_H2-2 gene segment immediately adjacent to 23-mer RSS;

(ii) the engineered D_H gene segment comprises a human D_H2-8 gene segment immediately adjacent to 23-mer RSS;

(iii) the engineered D_H gene segment comprises a human D_H2-15 gene segment immediately adjacent to 23-mer RSS; or

(iv) any combination thereof, and

(c) at least one ~~or more~~ unrearranged human J_H gene ~~segments~~segment.

~~19~~7. The nucleotide molecule of claim ~~17~~5 or claim ~~18~~6, wherein:

(a) the at least one ~~or more~~ unrearranged human V_H gene ~~segments~~segment comprises from 5' to 3' an unrearranged human V_H1-2 gene segment and an unrearranged human V_H6-1 gene segment, wherein the nucleotide further comprises one or more functional rodent Adam6 genes between the unrearranged human V_H1-2 gene segment and the unrearranged human V_H6-1 gene segment, and wherein the unrearranged human V_H1-2 gene segment, the one or more functional rodent Adam6 genes, and the unrearranged human V_H6-1 gene segment are contiguous~~.~~;

~~20. The nucleotide molecule of any~~ (b) the at least one ~~of claims 17-19, wherein the one or more~~ unrearranged human V_H gene ~~segments~~segment comprises the full repertoire of functional unrearranged human V_H gene segments ~~spanning between and including the unrearranged human V_H3-~~

~~74 and unrearranged human V_H1-6 gene segments~~, optionally wherein the full repertoire of functional unrearranged human V_H gene segments is in germline configuration~~.~~; and/or

~~21. The nucleotide molecule of any one of claims 17-20, wherein~~ (c) the at least one ~~or more~~ unrearranged human J_H gene ~~segments~~segment comprises an unrearranged human J_H6 gene segment~~.~~, optionally wherein:

~~22. The nucleotide molecule of claim 21, wherein~~ (i) the at least one ~~or more~~ unrearranged human J_H gene ~~segments~~segment comprises an unrearranged human J_H4 gene segment, an unrearranged human J_H5 gene segment, and the unrearranged human J_H6 gene segment~~.~~; and/or

~~23. The nucleotide molecule of claim 22,~~ (ii) wherein the at least one ~~or more~~ unrearranged human J_H gene ~~segments~~segment comprises an unrearranged human J_H1 gene segment, an unrearranged human J_H2 gene segment, an unrearranged human J_H3 gene segment, ~~the~~an unrearranged human J_H4 gene segment, ~~the~~an unrearranged human J_H5 gene segment, and ~~the~~an unrearranged human J_H6 gene segment, further optionally wherein the unrearranged human J_H1~~,~~ gene segment, the unrearranged J_H2~~,~~ gene segment, the unrearranged J_H3~~,~~ gene segment, the unrearranged J_H4~~,~~ gene segment, the unrearranged J_H5 gene segment, and the unrearranged J_H6 gene ~~segments~~segment are in germline configuration.

~~24.~~8. The nucleotide molecule of any one of claims 1-~~23~~7, further comprising:

(a) at its 3'-end, a heavy chain immunoglobulin constant (C_H) region ~~(C_H)~~ or a portion thereof~~.~~;

~~25. The nucleotide molecule of claim 24, wherein the C_H is~~ (b) at its 3'-end, a rodent C_H region or a portion thereof~~.~~;

~~26. The nucleotide molecule of claim 25, wherein the~~ (c) at its 3' end, a rodent C_H region or a portion thereof ~~comprises~~comprising a rodent intronic enhancer region and a rodent IgM gene~~.~~; and/or

~~27. The nucleotide molecule of any one of claims 1-26, further comprising a~~ (d) a drug selection cassette~~,~~

~~wherein the drug selection cassette is~~ located upstream of the engineered D_H gene segment ~~operably linked to a 23-mer RSS, and~~

~~wherein the drug selection cassette is,~~ optionally wherein the drug selection cassette is flanked by one or more site-specific recombination sites.

~~28.~~9. A targeting vector comprising a nucleotide molecule of any one of claims 1-~~27~~8, comprising 5'- and 3'- homology arms configured to allow homologous recombination with an immunoglobulin heavy chain sequence~~,~~ at an endogenous rodent immunoglobulin heavy chain locus, optionally wherein the immunoglobulin heavy chain sequence ~~is at an endogenous rodent immunoglobulin heavy chain locus,~~ comprises a human or humanized immunoglobulin heavy chain variable region~~, or a combination thereof~~.

~~29~~10. A method of modifying an immunoglobulin heavy chain variable region to engineer D_H-D_H recombination, the method comprising:

(a) obtaining an immunoglobulin heavy chain variable ~~region~~chain sequence comprising a D_H region comprising one or more unrearranged D_H gene segments, each of which unrearranged D_H gene ~~segments are~~segment is flanked on ~~one~~its 5' side by a first 12-mer RSS and on ~~the other~~its 3' side by ~~another12~~a second 12-mer RSS, ~~and~~

~~modifying the D_H region to further comprise at least one D_H gene segment operably linked to a 23-mer RSS.~~

~~30. The method of claim 29,~~ optionally wherein the one or more unrearranged D_H gene segments comprises ~~one or more~~ at least one unrearranged human D_H gene segment, further optionally wherein the at least one unrearranged human D_H gene ~~segments.~~

~~31. The method of claim 30, wherein the one or more unrearranged D_H gene segments~~ segment comprises all the functional ~~germline~~ human D_H gene segments spanning between~~,~~ and including~~, the germline~~ an unrearranged human D_H1-1 gene segment and ~~the germline~~an unrearranged human ~~D_H7~~D_H 7-27 gene ~~segments~~segment, optionally wherein all the functional human D_H gene segments are in germline configuration~~.~~, and

~~32. The method of any one of claims 29-31, wherein modifying comprises replacing~~

~~(a) at least one of the one~~(b) engineering the D_H region to further comprise at least one engineered D_H gene segment, wherein the engineered D_H gene segment comprises a D_H gene segment immediately adjacent to a 23-mer RSS,

wherein, in the resulting engineered D_H region, the engineered D_H gene segment and at least one of the one or more unrearranged D_H gene segments are operably linked and able to join in a D_H-D_H recombination event according to the 12/23 rule,

optionally wherein the resulting engineered D_H region comprises only human D_H gene segments.

11. The method of claim 10, wherein the D_H region of claim 10(a) comprises two or more unrearranged D_H gene segments, each of which is flanked on its 5’ side by a first 12-mer RSS and on its 3’ side by a second 12-mer RSS, and

wherein engineering comprises

(a) replacing at least one of the two or more unrearranged D_H gene segments with ~~a~~ the engineered D_H gene segment ~~operably linked to a 23-mer RSS,~~as defined in claim 10(b),

(b) replacing the first 12-mer RSS or the second 12-mer RSS of at least one of the two ~~12-mer RSS flanking an~~or more unrearranged D_H gene ~~segment~~segments with a 23-mer RSS, or

(c) a combination of (a) and (b~~.~~),

~~33. The method of claim 32~~ optionally, wherein the immunoglobulin heavy chain variable ~~region~~chain sequence further comprises a J_H region comprising at least one unrearranged J_H gene segment,

wherein the J_H region is operably linked to the D_H region, and

wherein replacing at least one of the ~~one~~two or more unrearranged D_H gene segments with the engineered D_H gene segment ~~operably linked to the 23-mer RSS~~ as defined in claim 10(b) comprises deleting the at least one unrearranged J_H gene segment ~~comprised in the J_H region.~~.

~~34. The method of claim 33,~~ further optionally wherein the J_H region comprises a full repertoire of human germline J_H gene segments comprising ~~a~~an unrearranged human ~~germline~~ J_H1 gene segment, ~~a~~an unrearranged human ~~germline~~ J_H2 gene segment, ~~a~~ an unrearranged human ~~germline~~ J_H3 gene segment, ~~a~~an unrearranged human ~~germline~~ J_H4 gene segment, ~~a~~an unrearranged human ~~germline~~ J_H5 gene segment, and ~~a~~an unrearranged human ~~germline~~ J_H6 gene segment, optionally wherein the unrearranged human ~~germline~~ J_H1~~,~~ gene segment, the unrearranged human J_H2~~,~~ gene segment, the unrearranged human J_H3~~,~~ gene segment, the unrearranged human J_H4~~,~~ gene segment, the unrearranged human J_H5 gene segment, and the unrearranged human J_H6 gene ~~segments~~segment are in germline configuration, and

wherein deleting at least one germline J_H gene segment ~~comprised in the J_H region~~ comprises deleting the unrearranged human J_H1~~,~~ gene segment, the unrearranged human J_H2 gene segment, and the unrearranged human J_H3 gene ~~segments~~segment, and optionally further deleting the unrearranged human J_H4 gene segment and the unrearranged human J_H5 gene ~~segments~~segment.

~~35~~

12. The method of ~~any one of~~ claim ~~29 to 34~~10 or 11, wherein:

(a) the ~~at least one~~engineered D_H gene segment ~~operably linked to a 23-mer RSS~~as defined in claim 10(b) comprises

~~(a~~ (i) a human D_H3-3 gene segment ~~operably linked~~immediately adjacent to ~~a~~the 23-mer RSS, optionally wherein the 23-mer RSS is ~~contiguous~~immediately adjacent to the 5'-end of the D_H3-3 gene segment,

~~(b~~ (ii) a human D_H2-2 gene segment ~~operably linked~~immediately adjacent to ~~a~~the 23-mer RSS, optionally wherein the 23-mer RSS is ~~contiguous~~immediately adjacent to the 3'-end of the D_H2-2 gene segment,

~~(c~~ (iii) a human D_H2-8 gene segment ~~operably linked~~immediately adjacent to ~~a~~the 23-mer RSS, optionally wherein the 23-mer RSS is ~~contiguous~~immediately adjacent to the 3'-end of the D_H2-8 gene segment,

~~(d~~ (iv) a human D_H2-15 gene segment ~~operably linked~~immediately adjacent to ~~a~~the 23-mer RSS, optionally wherein the 23-mer RSS is ~~contiguous~~immediately adjacent to the 3'-end of the D_H2-15 gene segment, or

~~(e~~ (v) any combination of ~~(a)-(d).~~i)-(iv);

~~36. The method of any one of claims 32-35, wherein the at least one unrearranged D_H gene segment that is replaced~~(b) engineering comprises replacing the most 3' unrearranged D_H gene segment of the D_H region~~.~~

~~37. The method of any one of claims 32-36, wherein the at least one unrearranged~~ as defined in claim 10(a) with the engineered D_H gene segment ~~that is replaced~~as defined in claim 10(b); and/or

(c) engineering comprises ~~a~~replacing at least one of the one or more unrearranged D_H gene ~~segment~~segments that corresponds to the ~~at least one~~engineered D_H gene segment ~~operably linked to a 23-mer RSS.~~as defined in claim 10(b) with the engineered D_H gene segment as defined in claim 10(b).

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13. An immunoglobulin heavy chain locus comprising the nucleotide molecule of any one of claims 1-~~27~~8, the targeting vector of claim ~~28~~9, or the immunoglobulin heavy chain variable region modified according to the method of any one of claims ~~29-37~~10-12.

~~39~~14. A rodent genome comprising the ~~nucleic acid~~nucleotide molecule of any one of claims 1-~~27~~8, the targeting vector of claim ~~28~~9, the immunoglobulin heavy chain variable region modified

according to the method of any one of claims ~~29-37~~10-12, or the immunoglobulin heavy chain locus of claim ~~38~~13, wherein the rodent genome is optionally a rodent germline genome.

~~40. A~~15. The rodent ~~germline~~ genome ~~comprising~~of claim 14, wherein the rodent genome comprises an immunoglobulin heavy chain variable region that comprises:

(~~i~~a) unrearranged ~~heavy chain variable (~~V_H~~)~~ gene segments,

(~~ii) an~~b) the engineered ~~heavy chain variable~~D_H region ~~diversity (D_H) region,~~, optionally wherein the engineered D_H region comprises ~~one or more germline~~only human D_H gene segments, and ~~one or more D_H gene segments operably linked to a 23-mer recombination signal sequence (RSS), and~~

(~~iii~~c) unrearranged ~~heavy chain joining (~~J_H~~)~~ gene segments,

wherein (~~i) (iii~~a)-(c) are in operable linkage such that, upon recombination, the immunoglobulin heavy chain variable region comprises a rearranged heavy chain variable region sequence encoding an immunoglobulin heavy chain variable domain, optionally wherein the rearranged heavy chain variable region sequence is formed after a $V_H(D_H\text{-}D_H)J_H$ recombination event~~.~~,

~~41. The rodent germline genome of claim 40,~~ optionally wherein:

(aa) the ~~one or more~~engineered D_H gene ~~segments operably linked to a 23-mer recombination signal sequence (RSS)~~ segment comprises a D_H segment ~~operably linked~~immediately adjacent to a 3' 23-mer RSS~~.~~;

~~42. The rodent germline genome of claim 40 or claim 41, wherein the one or more~~ (bb) the engineered D_H gene segments ~~operably linked to a 23-mer recombination signal sequence (RSS)~~ comprises a D_H gene segment ~~operably linked~~immediately adjacent to a 5' 23-mer RSS~~.~~;

~~43. The rodent germline genome of any one of claims 40-42, wherein~~ (cc) the immunoglobulin heavy chain variable region is a human immunoglobulin heavy chain variable region comprising only human V_H~~,~~ gene segments, human D_H gene segments, and human J_H gene segments~~.~~

~~44. The rodent germline genome of claim 43,~~ , optionally wherein the human immunoglobulin heavy chain variable region is operably linked to ~~an immunoglobulin heavy chain constant~~a C_H region~~.~~

~~45. The rodent germline genome of claim 44,~~ further optionally wherein the ~~immunoglobulin heavy chain constant~~C_H region is an endogenous ~~rodent immunoglobulin heavy chain constant~~C_H region, optionally at an endogenous ~~rodent~~ immunoglobulin heavy chain locus~~.~~;

~~46. The rodent germline genome of any one of claims 40-45, wherein~~ (dd) the unrearranged V_H gene segments comprise ~~a~~the full repertoire of functional ~~germline~~unrearranged human V_H gene segments ~~spanning between and including V_H3-74 to V_H6-1~~, optionally wherein the full repertoire of functional unrearranged human V_H gene segments is in germline configuration~~.~~;

~~47. The rodent germline genome of any one of claims 40-46, wherein~~ (ee) the unrearranged ~~heavy chain~~ J_H gene segments comprise a human J_H6 gene segment~~.~~; and/or

~~48. The rodent germline genome of any one of claims 40-47, wherein rodent germline~~ (ff) (i) the rodent genome lacks an endogenous Adam6 gene~~.~~; or

~~49. The~~ (ii) the rodent ~~germline~~ genome ~~of any one of claims 40-47,~~ further ~~comprising~~comprises one or more rodent Adam6 genes, optionally wherein:

the one or more rodent ~~endogenous~~ Adam6 genes comprises an endogenous rodent Adam6 gene~~.~~;

~~50. The rodent germline genome of claim 49, wherein~~ the one or more rodent Adam6 genes are inserted between two human V_H gene segments~~.~~,

~~51. The rodent germline genome of claim 50,~~ optionally wherein the one or more rodent Adam6 genes are inserted between ~~a~~an unrearranged human V_H1-2 gene segment and ~~a~~an unrearranged human V_H6-1 gene segment~~.~~

~~52. The rodent germline genome of claim 49, wherein~~ the one or more one or more rodent Adam6 genes are inserted in the place of a human Adam6 pseudogene~~.~~; and/or

~~53. The rodent germline genome of claim 49, wherein.~~ the one or more one or more rodent Adam6 genes are inserted between ~~a~~an unrearranged human V_H gene segment and ~~a~~an unrearranged human D_H gene segment.

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16. The rodent ~~germline~~ genome of ~~any one of claims 39-53,~~claim 14 or 15, wherein:

(a) the rodent genome further ~~comprising~~comprises a terminal deoxynucleotidyl transferase gene~~.~~;

~~55. The~~ (b) the rodent ~~germline~~ genome ~~of any one claims 39-54,~~is characterized in that it is homozygous for the engineered D_H region~~.~~

~~56. The~~ or the rodent ~~germline~~ genome ~~of any one claims 39-54,~~is characterized in that it is heterozygous for the engineered D_H region~~.~~; and/or

~~57. The~~ (c) the rodent ~~germline~~ genome ~~of any one of claims 39-56, wherein the rodent~~ is a rat genome or a mouse genome.

~~58~~17. A rodent ~~or rodent~~ cell comprising the rodent ~~germline~~ genome of any one of claims ~~39-57.~~

~~59. The rodent or rodent cell of claim 58,~~14 to 16, optionally wherein the rodent ~~or rodent~~ cell is a rat~~, a mouse, a rat~~ cell~~,~~ or a mouse cell.

~~60~~18. The rodent ~~or rodent~~ cell of claim ~~58 or claim 59~~17, wherein the rodent cell is a rodent embryonic stem cell.

~~61~~19. A rodent ~~or rodent embryo~~ generated from the rodent embryonic stem cell of claim ~~60~~18, wherein the rodent comprises the rodent genome in its germline.

~~62~~20. The rodent~~,~~ of claim 19, wherein:

(a) the rodent ~~embryo, or rodent cell of any one of claims 58-61,~~ further ~~comprising~~comprises a somatic genome that comprises a rearranged heavy chain $V_H(D_HA\text{-}D_HB)J_H$ coding sequence,

optionally wherein the rearranged heavy chain $V_H(D_HA\text{-}D_HB)J_H$ ~~gene segment~~coding sequence is operably linked to an IgM constant region gene sequence and/or wherein D_HA or D_HB is derived from the engineered D_H gene segment ~~operably linked to a 23-mer RSS~~ or a portion thereof and at least 9 base pairs of D_HA or D_HB is identical to at least 9 base pairs of the engineered D_H gene segment ~~operably linked to a 23-mer RSS or a portion thereof~~; and ~~each of.~~ /or

~~63. The rodent of any one of claims 58-62, wherein~~ (b) at least 95% of all rearranged heavy chain VDJ coding sequences in the rodent have a CDR3 length of at least 10 amino acids, optionally wherein at least 70% of all rearranged heavy chain VDJ gene sequences in the rodent have a CDR3 length of at least 11 amino acids, optionally wherein at least 15% of all rearranged heavy chain VDJ gene sequences in the rodent have a CDR3 length of at least 14 amino acids~~.~~

~~64. A rodent according to any one of claims 58-63,~~ further optionally characterized in that the rodent exhibits at least a at least a 3-fold increased frequency of D_H-D_H recombination as compared to the frequency of D_H-D_H recombination in a reference rodent.

~~65~~21. A method of making a rodent whose genome comprises an engineered D_H region, the method comprising

(A) generating ~~a~~the rodent from the embryonic stem cell of claim ~~60.~~18, or

~~66. A method of making a rodent whose genome comprises an engineered D_H region, the method comprising~~

~~(a)~~ (B)

(a) modifying the genome of a rodent embryonic stem cell to comprise a DNA fragment comprising one or more engineered D_H gene segments, each ~~operably linked~~comprising a D_H gene segment immediately adjacent to a 23-mer RSS, optionally wherein the DNA fragment comprises the nucleotide molecule of any one of claims 1-~~27~~8, the targeting vector of claim ~~28,~~9, the immunoglobulin heavy chain variable region modified according to the method of any one of claims ~~30-37~~10-12, or the immunoglobulin heavy chain of claim ~~38~~13, and

~~(b)~~ generating ~~a~~the rodent using the modified rodent embryonic stem cell of (B)(a).

~~67. A method of making a rodent whose genome an engineered D_H region, the method comprising~~

~~modifying an unrearranged D_H region of an immunoglobulin heavy chain variable region to comprise at least one D_H segments operably linked to a 23-mer RSS, wherein the unrearranged D_H region further comprises one or more germline D_H gene segments.~~

~~thereby making said rodent.~~

~~68.~~22. The method of claim ~~67, wherein modifying comprises replacing the one or more unrearranged human D_H gene segments, and optionally replacing one or more J_H gene segments, with the at least one D_H gene segment operably linked to a 23-mer RSS.~~

~~69. The method of claim 67 or claim 68, wherein the immunoglobulin heavy chain variable region is a human immunoglobulin heavy chain variable region, optionally wherein the human immunoglobulin heavy chain variable region comprises an unrearranged human immunoglobulin heavy chain V_H gene cluster comprising at least one V_H gene segment, an unrearranged human immunoglobulin heavy chain D_H region comprising one or more unrearranged human D_H gene segments, and an unrearranged human immunoglobulin heavy chain J_H gene cluster comprising at one unrearranged human J_H gene segment, wherein the unrearranged human immunoglobulin heavy chain D_H region is modified to comprise at least one D_H gene segment operably linked to a 23-mer RSS.~~

70. The method of claim 69, wherein the modifying step results in the immunoglobulin heavy chain variable region comprising

(i) the full repertoire of functional human V_H gene segments, optionally all the functional human V_H gene segments spanning between and including V_H3-74 to V_H6-1, optionally wherein all the functional V_H gene segments are positioned in germline configuration,

(ii) the full repertoire of unrearranged human D_H gene segments except for D_H7-27 which is replaced with the at least one D_H gene segment operably linked to a 23-mer RSS, and

(iii) at least an unrearranged human J_H6 gene segment, and optionally at least an unrearranged human J_H4 gene segment, an unrearranged human J_H5 gene segment, and an unrearranged human J_H6 gene segment.

71. The method of claim 70, wherein the at least one D_H gene segment operably linked to a 23-mer RSS comprises a D_H3-3 gene segment operably linked to a 5’ 23-mer RSS.

72. The method of claim 69, wherein the modifying step results in the immunoglobulin heavy chain variable region comprising

(i) a full repertoire of functional human V_H gene segments, optionally all functional V_H gene segments spanning between and including V_H3-74 to V_H6-1, optionally wherein all the functional V_H gene segments are positioned in germline configuration

(ii) the full repertoire of unrearranged human D_H gene segments except for the unrearranged D_H2-2, D_H2-8, and D_H2-15 gene segments, each of which are replaced with at least one D_H gene segment operably linked to a 23-mer RSS, and

(iii) the full repertoire of unrearranged human J_H gene segments, optionally an unrearranged human J_H1 gene segment, an unrearranged human J_H2 gene segment, an unrearranged human J_H3 gene segment, an unrearranged human J_H4 gene segment, an unrearranged human J_H5 gene segment, and an unrearranged human J_H6 gene segment.

~~73. The method of claim 72, wherein the at least one D_H gene segment operably linked to a 23-mer RSS comprises a human D_H2-2 gene segment operably linked to a 3' 23-mer RSS, a human D_H2-8 gene segment operably linked to a 3' 23-mer RSS, or a human D_H2-15 gene segment operably linked to a 3' 23-mer RSS, optionally wherein the unrearranged D_H2-2, D_H2-8, and D_H2-15 gene segments are respectively replaced with the human D_H2-2 gene segment operably linked to a 3' end 23-mer RSS, the human D_H2-8 gene segment operably linked to a 3'-end 23-mer RSS, and the human D_H2-15 gene segment operably linked to a 3'-end 23-mer RSS.~~

~~74. The method of claim of any one of claims 67-73, wherein the immunoglobulin heavy chain variable region~~

~~(a) further comprises one or more rodent Adam6 genes, optionally wherein the one or more rodent Adam6 genes are located between two unrearranged V_H gene segments, optionally between an unrearranged human V_H1-2 gene segment and an unrearranged human V_H6-1 gene segment, and/or~~

~~(b) is operably linked to an immunoglobulin heavy chain constant region, optionally wherein the immunoglobulin heavy chain constant region is an endogenous rodent immunoglobulin heavy chain constant region, optionally an endogenous rodent immunoglobulin heavy chain constant region at an endogenous immunoglobulin heavy chain locus.~~

~~75. The method of any one of claims 65-74~~21, wherein the rodent is a rat or a mouse.

~~76~~23. A method of producing an antibody or obtaining a nucleic acid encoding same, the method comprising

immunizing a rodent with an antigen, which rodent comprises a germline genome comprising an engineered D_H region that comprises one or more engineered D_H gene segments that are each ~~operably linked~~immediately adjacent to a 23-mer RSS, optionally wherein the rodent is the rodent ~~accordingly~~according to any one of claims ~~58-64~~19 to 20 or made according to claim 21, and

allowing the rodent to produce an immune response to the antigen including an antibody, or nucleic acid encoding same, that binds the antigen~~.~~.

~~77. The~~optionally wherein:

(a) the method ~~of claim 76,~~is one further comprising recovering the antibody, or nucleic acid encoding same, from the rodent or a rodent cell~~.~~, wherein the rodent cell is a B cell or a hybridoma;

~~78. The method of claim 76, wherein the rodent cell is a B cell or a hybridoma.~~

~~79. The method of any one of claims 76-78, wherein~~ (b) one or more engineered D_H gene segments that are each ~~operably linked~~immediately adjacent to a 23-mer RSS comprises a D_H gene segment operably linked to a 5' 23-mer RSS~~.~~;

~~80. The method of any one of claims 76-79, wherein the~~ (c) one or more engineered D_H gene segments that are each ~~operably linked~~immediately adjacent to a 23-mer RSS comprises a D_H gene segment operably linked to a 3' 23-mer RSS~~.~~; and/or

~~81. The method of any one of claims 76-80, wherein~~ (d) the rodent is a rat or a mouse.

~~82. A rodent genome, nucleic acid or immunoglobulin locus comprising a rearranged heavy chain $V_H(D_HA$-$D_HB)J_H$ coding sequence operably linked to a rodent heavy chain constant region gene sequence, optionally wherein the rearranged heavy chain is somatically hypermutated and/or determined to be a $V_H(D_HA$-$D_HB)J_H$ coding sequence since each of D_HA and D_HB respectively shows 40% identity to a first and second germline D_H gene segment, with a maximum of 1 nucleotide mutation.~~

~~83. The rodent genome, nucleic acid or immunoglobulin locus of claim 82, wherein the D_HB gene segment is derived from a human germline D_H3-3 gene segment.~~

~~84. The rodent genome, nucleic acid or immunoglobulin locus of claim 82, wherein the D_HA gene segment is derived from a human germline D_H2-2, D_H2-8 or D_H2-15 gene segment.~~

~~85. The rodent genome, nucleic acid or immunoglobulin locus of any one of claims 82-84, wherein the rearranged heavy chain $V_H(D_HA\text{-}D_HB)J_H$ coding sequence encodes a CDR3 length of over 20 amino acids.~~

~~86. The rodent genome, nucleic acid or immunoglobulin locus of any one of claims 82-85, wherein the J_H gene segment is derived from a human germline J_H6 gene segment.~~

~~87. A rodent or rodent cell comprising the genome, nucleic acid, or immunoglobulin locus of any one of claims 82-86.~~

~~88. The rodent or rodent cell of claim 87, wherein the rodent is a rat or a mouse or the rodent cell is a rat cell or a mouse cell.~~

~~89. The rodent or rodent cell of claim 87 or claim 88, wherein the rodent cell is a rodent B cell.~~

~~90. A hybridoma comprising the rodent B cell of claim 89 fused with a myeloma cell.~~