We claim:

1. A method for targeted modification of a genomic locus of interest in a pluripotent rat cell, comprising~~.~~:

(a)~~.~~ introducing into the pluripotent rat cell a large targeting vector (LTVEC) comprising an insert nucleic acid flanked ~~with~~by a 5' ~~rat~~ homology arm complementary to a first nucleic acid sequence at the genomic locus of interest and a 3' ~~rat~~ homology arm complementary to a second nucleic acid sequence at the genomic locus of interest, wherein the ~~sum total of the~~ 5' homology arm is at least 5 kb and the 3' homology ~~arms~~arm is at least ~~10~~5 kb ~~but less than 150kb~~; and

(b)~~.~~ identifying a genetically modified pluripotent rat cell comprising ~~the~~a targeted genetic modification at the genomic locus of interest,

wherein the targeted genetic modification is capable of being transmitted through the germline~~.~~, and

wherein the pluripotent rat cell to be used for targeted modification of the genomic locus of interest is obtainable by culturing isolated rat embryonic stem cells on a feeder cell layer that is not modified to express leukemia inhibitory factor (LIF) with a medium comprising about 50 U/mL to about 150 U/mL LIF and a combination of inhibitors consisting of a MEK pathway inhibitor and a GSK3 inhibitor.

2. The method of claim 1, wherein the ~~targeted genetic modification is biallelic.~~

~~3. The method of claim 1 or 2, wherein the~~ pluripotent rat cell is a rat embryonic stem (ES) cell~~.~~

~~4. The method of claim 1, 2 or 3,~~, optionally wherein the ~~pluripotent~~ rat ES cell is derived from a DA strain or an ACI strain.

~~5.~~ 3. The method of ~~any one of claims~~claim 1~~-~~4 or 2, wherein the pluripotent rat cell~~-~~:

(i) is characterized by expression of at least one pluripotency marker ~~comprising~~ selected from the group consisting of Dnmt3L, Eras, Err-beta, Fbxo15, Fgf4, Gdf3, Klf4, Lef1, LIF receptor, Lin28, Nanog, Oct4, Sox15, Sox2, and Utf1~~, or a combination thereof.~~;

~~6. The method of any one of claims 1-4 wherein the pluripotent rat cell is characterized by one of more of the following characteristics:~~

~~(a) lack of~~(ii) lacks expression of one or more of the pluripotency markers ~~comprising~~ c-Myc, Ecat1, and~~/or~~ Rexo1;

(~~b) lack of~~iii) lacks expression of one or more of the mesodermal markers ~~comprising~~ Brachyury and~~/or~~ Bmpr2;

~~(c) lack of~~(iv) lacks expression of one or more of the endodermal markers ~~comprising~~ Gata6, Sox17, and~~/or~~ Sox7; or

(~~d) lack of~~v) lacks expression of one or more of the neural markers ~~comprising~~ Nestin and~~/or~~ Pax6.

~~7.~~ 4. The method of any one of claims 1-~~6~~3, wherein the ~~sum total~~concentration of LIF in the ~~5' and the 3' homology arms of the LTVEC~~medium is ~~from about 10kb to about 30kb, from~~between about ~~20kb~~75 U/mL to about ~~40kb, from~~125 U/mL, preferably between about ~~40kb~~90 U/mL to about ~~60kb, from~~110 U/mL, and more preferably about ~~60kb~~100 U/mL.

5. The method of any one of claims 1-4, wherein the MEK pathway inhibitor is MEK inhibitor PD0325901 and/or the GSK3 inhibitor is CHIR99021.

6. The method of claim 5, wherein the MEK pathway inhibitor is MEK inhibitor PD0325901 at a concentration of 0.8 µM to 1.2 µM, and the GSK3 inhibitor is CHIR99021 at a concentration of 2.5 µM to 3.5 µM.

7. The method of claim 6, wherein the concentration of LIF in the medium is about ~~80kb, from~~100 U/mL, the MEK pathway inhibitor is MEK inhibitor PD0325901 at a concentration of about ~~80kb to~~1 µM, and the GSK3 inhibitor is CHIR99021 at a concentration of about ~~100kb, from about 100kb to about 120kb, or from about 120kb to 150kb~~3 µM.

8. The method of claim 7, wherein the medium is a 2i medium that comprises: DMEM/F12 basal medium at a concentration of lx; neurobasal medium at a concentration of lx; penicillin/streptomycin at a concentration of 1%; L-Glutamine at a concentration of 4 mM; 2-mercaptoethanol at a concentration of 0.1 mM; N2 supplement at a concentration of 1x; B27 supplement at a concentration 1x; LIF at a concentration of 100U/mL; PD0325901 at a concentration of 1 μM, and CHIR99021 at a concentration of 3 μM.

9. The method of any one of claims 1-~~6~~8, wherein the target genomic locus:

(i) is an Interleukin-2 receptor gamma locus, an ApoE locus, a Rag1 locus, a Rag2 locus, or a Rag2/Rag1 locus;

(ii) is an immunoglobulin locus,

optionally wherein the target genomic locus encodes a mammalian immunoglobulin heavy chain variable region amino acid sequence, or

optionally wherein the target genomic locus comprises a genomic DNA sequence that encodes a mammalian immunoglobulin light chain variable region amino acid sequence, optionally wherein the genomic DNA sequence comprises an unrearranged mammalian λ and/or κ light chain variable region nucleic acid sequence; or

(iii) is a T cell receptor locus, optionally wherein the T cell receptor locus is a T cell receptor alpha locus.

10. The method of any one of claims 1-9, wherein:

(i) the LTVEC is from about 20 kb to about 400 kb;

(ii) the 5' homology arm ranges from about 5 kb to about 100 kb or the 3' homology arm ranges from about 5 kb to about 100 kb;

(iii) the sum total of the 5' and the 3' homology arms of the LTVEC is from about ~~16Kb~~10 kb to about ~~150Kb.~~ 150 kb; or

~~9~~(iv) the sum total of the 5' and the 3' homology arms is at least 10 kb.

11. The method of any one of claims 1-~~8~~10, wherein the insert nucleic acid:

(i) comprises a polynucleotide of interest comprising a genomic nucleic acid

sequence that encodes a human immunoglobulin heavy chain variable region amino acid sequence;

(ii) comprises a polynucleotide of interest comprising a genomic nucleic acid sequence that encodes a human immunoglobulin light chain variable region amino acid sequence;

(iii) comprises a polynucleotide of interest comprising a polynucleotide encoding at least a region of a T cell receptor, optionally wherein the T cell receptor is a T cell receptor alpha; or

(iv) comprises a polynucleotide of interest comprising at least one disease allele.

12. The method of any one of claims 1-11, wherein the insert nucleic acid:

(i) is from a human;

(ii) comprises a knock-in allele of at least one exon of an endogenous gene;

(iii) comprises a regulatory element;

(iv) comprises a conditional allele;

(v) comprises a nucleic acid flanked by site-specific recombination target sequences, optionally wherein the site-specific recombination target sequences flank a polynucleotide encoding a selection marker and/or a reporter gene;

(vi) comprises a polynucleotide encoding a selection marker, optionally wherein the insert nucleic acid comprises a self-deleting selection cassette; or

(vii) comprises a reporter gene operably linked to a promoter.

13. The method of any one of claims 1-12, wherein the targeted genetic modification is biallelic.

14. The method of any one of claims 1-13, wherein the targeted genetic modification comprises:

(~~a) a~~ i) replacement of an endogenous rat nucleic acid sequence with a homologous or an orthologous nucleic acid sequence;

(~~b) a~~ ii) deletion of an endogenous rat nucleic acid sequence~~;~~

~~(c) a deletion of an endogenous rat nucleic acid sequence,~~

, optionally wherein the deletion ranges from about 5kb5 kb to about 10kb, from about 10kb to about 20kb, from about 20kb to about 40kb, from about 40kb to about 60kb, from about 60kb to about 80kb, from about 80kb to about 100kb, from about 100kb to about 150kb, or from about 150kb to about 200kb, from about 200kb to about 300kb, from about 300kb to about 400kb, from about 400kb to about 500kb, from about 500kb to about 1Mb, from about 1Mb to about 1.5Mb, from about 1.5Mb to about 2Mb, from about 2Mb to about 2.5Mb, or from about 2.5Mb to about 3Mb3 Mb;

(d) iii) an exogenous nucleic acid sequence ranging from about 5kb5 kb to about 10kb, from about 10kb to about 20kb, from about 20kb to about 40kb, from about 40kb to about 60kb, from about 60kb to about 80kb, from about 80kb to about 100kb, from about 100kb to about 150kb, from about 150kb to about 200kb, from about 200kb to about 250kb, from about 250kb to about 300kb, from about 300kb to about 350kb, or from about 350kb to about 400kb400 kb;

(e) (iv) an exogenous nucleic acid sequence comprising a nucleic acid sequence that is homologous or an orthologous to an endogenous rat nucleic acid sequence;

(f) v) a chimeric nucleic acid sequence comprising a human nucleic acid sequence and a rat nucleic acid sequence;

(g) vi) a conditional allele flanked withby site-specific recombinase target sequences; or,

(h) vii) a reporter gene operably linked to a promoter active in a rat cell.

10. 15. The method of any one of claims 1-914, wherein the genomic locus of interesttargeted genetic modification comprises:

(i) insertion of a firsthuman nucleic acid sequence that is complementaryhomologous or orthologous to the 5'a rat homology arm; and (ii) a second nucleic acid sequence that is complementary to the 3' rat homology arm.

11. The method of claim 10,at an endogenous genomic locus, optionally wherein the first and the second size of the insertion is from about 5 kb to about 500 kb;

(ii) replacement of the rat nucleic acid sequence is separated by at least 5kb

~~but less than 3Mb.~~ at the endogenous genomic locus with the homologous or orthologous human nucleic acid sequence, optionally wherein the size of the replacement is from about 5 kb to about 500 kb;

~~12. The method of claim 10, wherein the first and the second nucleic acid sequence is separated by at least 5kb but less than 10kb, at least 10kb but less than 20kb, at least 20kb but less than 40kb, at least 40kb but less than 60kb, at least 60kb but less than 80kb, at least about 80kb but less than 100kb, at least 100kb but less than 150kb, or at least 150kb but less than 200kb, at least about 200kb but less than about 300kb, at least about 300kb but less than about 400kb, at least about 400kb but less than about 500kb, at least about 500kb but less than about 1Mb, at least about 1Mb but less than about 1.5Mb, at least about 1.5Mb but less than about 2Mb, at least about 2Mb but less than about 2.5Mb, or at least about 2.5Mb but less than about 3Mb.~~

~~13~~(iii) a chimeric nucleic acid sequence comprising a human nucleic acid sequence and a rat nucleic acid sequence; or

(iv) a combination thereof.

16. The method of any one of ~~claim~~claims 1~~-12~~15, wherein introducing step (a) further comprises introducing a ~~second~~ nucleic acid encoding a nuclease agent that promotes a homologous recombination between the ~~targeting construct~~LTVEC and the genomic locus of interest in the pluripotent rat cell.

~~14~~17. The method of claim ~~13~~16, wherein the nuclease agent comprises~~-~~:

(~~a) a chimeric protein comprising~~ i) a zinc finger~~-based DNA binding domain fused to a FokI endonuclease~~ nuclease; or~~,~~

(~~b) a chimeric protein comprising a~~ (ii) a Transcription Activator-Like Effector Nuclease (TALEN~~) fused to a FokI endonuclease.~~ ).

~~15.~~ 18. The method of ~~any one of claims 1-12~~claim 16, wherein ~~introducing step (a) further~~the nuclease agent comprises ~~introducing into the pluripotent rat cell:~~

~~(i) a first expression construct comprising a first promoter operably linked to a first nucleic acid sequence encoding a Clustered Regularly Interspaced Short Palindromic Repeats (~~a CRISPR~~) associated (~~/Cas~~) protein,~~

~~(ii) a second expression construct comprising a second promoter operably linked to a genomic target sequence linked to~~ system comprising a Cas9 nuclease and a guide RNA (gRNA~~),~~

~~wherein the genomic target sequence is immediately flanked on the 3' end by a Protospacer Adjacent Motif (PAM) sequence.~~

~~16. The method of claim 15, wherein the genomic locus of interest comprises the nucleotide sequence of SEQ ID NO: 1.~~

~~17. The method of claim 15 or 16, wherein the gRNA comprises a third nucleic acid sequence encoding a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) RNA (~~) comprising a fused crRNA~~) and a trans-activating CRISPR RNA (~~-tracrRNA~~).~~, optionally wherein:

~~18. The method of claim 15, 16 or 17, wherein the Cas protein is Cas9.~~

~~19. The method of claim 15, 16, 17, or 18, wherein~~ (i) the gRNA comprises:

~~(a) the~~(I) a chimeric RNA of the nucleic acid sequence of SEQ ID NO: 2; or

(II) a chimeric RNA of the nucleic acid sequence of SEQ ID NO: ~~2; or,~~

3;

~~(b) the chimeric RNA of the nucleic acid sequence of SEQ ID NO: 3.~~

~~20. The method of claim 17, wherein~~ (ii) the crRNA comprises SEQ ID NO: 4; SEQ ID NO: 5; or SEQ ID NO: 6~~.~~; or

21. The method of claim 17, wherein (iii) the tracrRNA comprises SEQ ID NO: 7 or SEQ ID NO: 8.

22. A modified rat genomic locus comprising:

(i) an insertion of a homologous or orthologous human nucleic acid sequence;

(ii) a replacement of an endogenous rat nucleic acid sequence with the homologous or orthologous human nucleic acid sequence; or

(iii) a combination thereof,

wherein the modified rat genomic locus is capable of being transmitted through the germline.

23. The modified rat genomic locus of claim 22, wherein the size of the insertion or replacement is from about 5kb to about 400kb.

24. The rat genomic locus of claim 22, wherein the size of the insertion or replacement is from about 5kb to about 10kb, from about 10kb to about 20kb, from about 20kb to about 40kb, from about 40kb to about 60kb, from about 60kb to about 80kb, from about 80kb to about 100kb, from about 100kb to about 150kb, from about 150kb to about 200kb, from about 200kb to about 250kb, from about 250kb to about 300kb, from about 300kb to about 350kb, or from about 350kb to about 400kb.

25. A method for making a humanized rat, comprising:

(a) targeting a genomic locus of interest in a pluripotent rat cell with a targeting construct comprising a human nucleic acid to form a genetically modified pluripotent rat cell;

(b) introducing the genetically modified pluripotent rat cell into a host rat embryo; and

(c) gestating the host rat embryo in a surrogate mother; wherein the surrogate mother produces rat progeny comprising a modified genomic locus that comprises:

(i) an insertion of a human nucleic acid sequence;

(ii) a replacement of the rat nucleic acid sequence at the genomic locus of interest with a homologous or orthologous human nucleic acid sequence;

~~(iii) a chimeric nucleic acid sequence comprising a human and a rat nucleic acid sequence; or~~

~~(iv) a combination thereof,~~

~~wherein the modified genomic locus is capable of being transmitted through the germline.~~

~~26. The method of claim 25, wherein the targeting construct is a large targeting vector (LTVEC), and the sum total of the 5' and the 3' homology arms of the LTVEC is at least 10 kb but less than 150kb.~~

~~27. The method of claim 26, wherein the sum total of the 5' and the 3' homology arms of the targeting construct is from about 10kb to about 30kb, from about 20kb to 40kb, from about 40kb to about 60kb, from about 60kb to about 80kb, or from about 80kb to about 100kb, from about 100kb to about 120kb, or from about 120kb to 150kb.~~

~~28. The method of claim 25, 26 or 27, wherein the human nucleic acid sequence is at least 5kb but less than 400kb.~~

~~29. The method of claim 25, 26, or 27, wherein the human nucleic acid sequence is at least 5kb but less than 10kb, at least 10kb but less than 20kb, at least 20kb but less than 40kb, at least 40kb but less than 60kb, at least 60kb but less than 80kb, at least about 80kb but less than 100kb, at least 100kb but less than 150kb, at least 150kb but less than 200kb, at least 200kb but less than 250kb, at least 250kb but less than 300kb, at least 300kb but less than 350kb, or at least 350kb but less than 400kb.~~

~~30~~. 19. The method of any one of claims ~~25-29~~1-18, wherein the ~~pluripotent rat cell~~introducing step is ~~a rat embryonic stem (ES) cell.~~ mediated by electroporation.

~~31~~20. The method of any one of claims ~~25-30, wherein the pluripotent rat cell is derived from a DA strain or an ACI strain.~~

32. The method of any one of claims 25-31, wherein the pluripotent rat cell is characterized by expression of at least one pluripotency marker comprising Dnmt3L, Eras, Err-beta, Fbxo15, Fgf4, Gdf3, Klf4, Lef1, LIF receptor, Lin28, Nanog, Oct4, Sox15, Sox2, Utf1, or a combination thereof.

33. The method of any one of claim 25-31, wherein the pluripotent rat cell is characterized by one or more of the following features:

(a) lack of expression of one or more pluripotency markers comprising c-Myc, Ecat1, and/or Rexo1;

(b) lack of expression of one or more mesodermal markers comprising Brachyury and/or Bmpr2;

(c) lack of expression of one or more endodermal markers comprising Gata6, Sox17, and/or Sox7; or

(d) lack of expression of one or more neural markers comprising Nestin and/or Pax6.

34. A modified rat comprising a humanized genomic locus, wherein the humanized genomic locus comprises:

(i) an insertion of a homologous or orthologous human nucleic acid sequence;

(ii) a replacement of a rat nucleic acid sequence at an endogenous genomic locus with a homologous or orthologous human nucleic acid sequence;

(iii) a chimeric nucleic acid sequence comprising a human and a rat nucleic acid sequence or,

(iv) a combination thereof,

wherein the humanized genomic locus is capable of being transmitted through the germline.

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35. A rat or rat cell comprising a targeted genetic modification in its genomic locus,

wherein the genomic locus is an Interleukin-2 receptor gamma locus, an ApoE locus, a Rag1 locus, a Rag2 locus, or a Rag2/Rag1 locus,

wherein the targeted genetic modification comprises:

(a) a deletion of an endogenous rat nucleic acid sequence at the genomic locus;

(b) an insertion of a homologous nucleic acid, an orthologous nucleic acid, or a chimeric nucleic acid comprising a human and a rat nucleic acid sequence, or

(c) a combination thereof,

wherein the targeted genetic modification is transmissible through the germline of the rat or a rat propagated from the rat cell.

36. The rat or rat cell of claim 35, wherein

(a) the deletion of the endogenous rat nucleic acid at the genomic locus is at least about 10kb; or,

(b) the deletion of the endogenous rat nucleic acid at the genomic locus is from about 5kb to about 10kb, from about 10kb to about 20kb, from about 20kb to about 40kb, from about 40kb to about 60kb, from about 60kb to about 80kb, from about 80kb to about 100kb, from about 100kb to about 150kb, or from about 150kb to about 200kb, from about 200kb to about 300kb, from about 300kb to about 400kb, from about 400kb to about 500kb, from about 500kb to about 1Mb, from about 1Mb to about 1.5Mb, from about 1.5Mb to about 2Mb, from about 2Mb to about 2.5Mb, or from about 2.5Mb to about 3Mb;

(c) the insertion of the exogenous nucleic acid sequence at the genomic locus is at least about 5 kb; or,

(d) theinsertion of the exogenous nucleic acid sequence at the genomic locus is from about 5kb to about 10kb, from about 10kb to about 20kb, from about 20kb to about 40kb, from about 40kb to about 60kb, from about 60kb to about 80kb, from about 80kb to about 100kb, from about 100kb to about 150kb, from about 150kb to about 200kb, from about 200kb to about 250kb, from about 250kb to about 300kb, from about 300kb to about 350kb, or from about 350kb to about 400kb.

37. The rat or rat cell of claim 35 or 36, wherein

(a) the targeted genetic modification at the Interleukin-2 receptor gamma locus results in a decrease in or absence of Interleukin-2 receptor gamma protein activity;

(b) the targeted genetic modification at the ApoE locus results in a decrease in or absence of ApoE protein activity;

(c) the targeted genetic modification at the Rag1 locus results in a decrease in or

absence of Rag1 protein activity;

(d) the targeted genetic modification at the Rag2 locus results in a decrease in or absence of Rag2 protein activity; or,

(e) the targeted genetic modification at the Rag2/Rag1 locus results in a decrease in or absence of Rag2 protein activity and Rag1 activity.

38. The rat or rat cell of claim 35, 36, or 37, wherein the targeted genetic modification of the Interleukin-2 receptor gamma locus comprises:

(a) a deletion of the entire rat Interleukin-2 receptor gamma coding region or a portion thereof;

(b) a replacement of the entire rat Interleukin-2 receptor gamma coding region or a portion thereof with a human Interleukin-2 receptor gamma coding region or a portion thereof;

(c) a replacement of an ecto-domain of the rat Interleukin-2 receptor gamma coding region with the ecto-domain of a human Interleukin-2 receptor gamma; or,

(d) at least a 3 kb deletion of the Interleukin-2 receptor gamma locus.

39. The rat or rat cell of any one of claims 35-37, wherein the targeted genetic modification of the ApoE locus comprises:

(a) a deletion of the entire ApoE coding region or a portion thereof; or,

(b) at least a 1.8 kb deletion of the ApoE locus comprising the ApoE coding region.

40. The rat or rat cell of any one of claims 35-37, wherein the targeted genetic modification of the Rag2 locus comprises:

(a) a deletion of the entire Rag2 coding region or a portion thereof;

(b) at least a 5.7 kb deletion of the Rag2 locus comprising the Rag2 coding region.

41. The rat or rat cell of any one of claims 35-37, wherein the targeted genetic modification of the Rag2/Rag1 locus comprises:

(a) a deletion of the entire Rag2 coding region or a portion thereof and a deletion of the entire Rag1 coding region or portion thereof; or,

(d) a deletion of at least 16 kb of the Rag2/Rag1 locus comprising the Rag2 coding

~~region.~~

~~42. The rat or rat cell of any one of claim 35-41, wherein the targeted genetic modification comprises an insertion of an expression cassette comprising a selective marker at the Interleukin-2 receptor gamma locus, the ApoE locus, the Rag1 locus, the Rag2 locus, or the Rag2/Rag1 locus.~~

~~43. The rat or rat cell of any one of claims 42, wherein the expression cassette comprises a lacZ gene operably linked to the endogenous promoter at the genomic locus and a human ubiquitin promoter operably linked to a selective marker.~~

~~44. The rat or rat cell of any one of claims 35-43, wherein the targeted genetic modification in the Interleukin-2 receptor gamma locus, the ApoE locus, the Rag1 locus, the Rag2 locus or the Rag2/Rag1 locus comprises the insertion of a self-deleting selection cassette.~~

~~45. The rat or rat cell of claim 44, wherein the self-deleting selection cassette comprises a selective marker gene operably linked to a promoter active in the rat cell and a recombinase gene operably linked to a male germ cell-specific promoter, wherein the self-deleting cassette is flanked by recombination recognition sites recognized by the recombinase.~~

~~46. The rat or rat cell of claim 45, wherein~~

~~(a) the male germ cell-specific promoter is a Protamine-1 promoter; or,~~

~~(b) the recombinase gene encodes Cre, and the recombination recognition sites are loxP sites.~~

~~47. The rat or rat cell of any one of claims 35-46, wherein the insertion of the exogenous nucleic acid sequence at the genomic locus comprises a reporter nucleic acid operably linked to an endogenous Interleukin-2 receptor gamma promoter, an endogenous ApoE promoter, an endogenous Rag1 promoter, or an endogenous Rag2 promoter.~~

~~48. The rat or rat cell of claim 47, wherein the reporter nucleic acid encodes a reporter~~

~~comprising β-galactosidase, mPlum, mCherry, tdTomato, mStrawberry, J-Red, DsRed, mOrange, mKO, mCitrine, Venus, YPet, enhanced yellow fluorescent protein (EYFP), Emerald, enhanced green fluorescent protein (EGFP), CyPet, cyan fluorescent protein (CFP), Cerulean, T-Sapphire, luciferase, alkaline phosphatase, or a combination thereof.~~

~~49. The rat cell of any one of claims 35-48, wherein the rat cell is a pluripotent rat cell or a rat embryonic stem (ES) cell.~~

~~50. The rat cell of claim 49, wherein the pluripotent rat cell or the rat embryonic stem (ES) cell~~

~~(a) is derived from a DA strain or an ACI strain;~~

~~(b) is characterized by expression of at least one pluripotency marker comprising Dnmt3L, Eras, Err-beta, Fbxo15, Fgf4, Gdf3, Klf4, Lef1, LIF receptor, Lin28, Nanog, Oct4, Sox15, Sox2, Utf1, or a combination thereof; or~~

~~(c) is characterized by one or more of the following characteristics:~~

~~(i) lack of expression of one or more pluripotency markers comprising c-Myc, Ecat1, and/or Rexo1;~~

~~(ii) lack of expression of mesodermal markers comprising Brachyury and/or Bmpr2;~~

~~(iii) lack of expression of one or more endodermal markers comprising Gata6, Sox17 and/or Sox7; or~~

~~(iv) lack of expression of one or more neural markers comprising Nestin and/or Pax6.~~

~~51. A method for modifying a target genomic locus in an Interleukin-2 receptor gamma locus, an ApoE locus, a Rag1 locus, a Rag2 locus or a Rag2/Rag1 locus in a pluripotent rat cell, the method comprising:~~

~~(a) introducing into the pluripotent rat cell a targeting vector comprising an insert nucleic acid flanked with 5' and 3' rat homology arms homologous to the target genomic locus,~~

~~(b) identifying a genetically modified pluripotent rat cell comprising a targeted genetic modification at the target genomic locus,~~

wherein the targeted genetic modification is capable of being transmitted through the germline of a rat propagated from the pluripotent rat cell.

52. The method of claim 51, wherein the targeting vector is a large targeting vector (LTVEC) wherein the sum total of the 5' and the 3' rat homology arms is at least about 10kb but less than about 150kb.

53. The method of claim 51 or 52, wherein introducing the targeting vector into the pluripotent rat cell leads to: (i) a deletion of an endogenous rat nucleic acid sequence at the target genomic locus; (ii) an insertion of an exogenous nucleic acid sequence at the target genomic locus; or (iii) a combination thereof.

54. The method of claim 53, wherein

(a) the deletion of the endogenous rat nucleic acid at the genomic locus is at least about 10 kb; or,

(b) the deletion of the endogenous rat nucleic acid at the genomic locus is from about 5kb to about 10kb, from about 10kb to about 20kb, from about 20kb to about 40kb, from about 40kb to about 60kb, from about 60kb to about 80kb, from about 80kb to about 100kb, from about 100kb to about 150kb, or from about 150kb to about 200kb, from about 200kb to about 300kb, from about 300kb to about 400kb, from about 400kb to about 500kb, from about 500kb to about 1Mb, from about 1Mb to about 1.5Mb, from about 1.5Mb to about 2Mb, from about 2Mb to about 2.5Mb, or from about 2.5Mb to about 3Mb;

(c) the insertion of the exogenous nucleic acid sequence at the genomic locus is at least about 5 kb; or,

(d) the insertion of the exogenous nucleic acid sequence at the genomic locus is from about 5kb to about 10kb, from about 10kb to about 20kb, from about 20kb to about 40kb, from about 40kb to about 60kb, from about 60kb to about 80kb, from about 80kb to about 100kb, from about 100kb to about 150kb, from about 150kb to about 200kb, from about 200kb to about 250kb, from about 250kb to about 300kb, from about 300kb to about 350kb, or from about 350kb to about 400kb.

55. The method of any one of claim 51-54, wherein

(a) the targeted genetic modification at the Interleukin-2 receptor gamma locus results in a decrease in or absence of Interleukin-2 receptor gamma protein activity;

(b) the targeted genetic modification at the ApoE locus results in a decrease in or absence of ApoE protein activity;

(c) the targeted genetic modification at the Rag1 locus results in a decrease in or absence of Rag1 protein activity;

(d) the targeted genetic modification at the Rag2 locus results in a decrease in or absence of Rag2 protein activity; or,

(e) the targeted genetic modification at the Rag2/Rag1 locus results in a decrease in or absence of Rag2 protein activity and i Rag1 protein activity.

56. The method of any one of claim 51-54, wherein the targeted genetic modification of the Interleukin-2 receptor gamma locus comprises

(a) a deletion of the entire rat Interleukin-2 receptor gamma coding region or a portion thereof;

(b) a replacement of the entire rat Interleukin-2 receptor gamma coding region or a portion thereof with a human Interleukin-2 receptor gamma coding region or a portion thereof;

(c) a replacement of an ecto-domain of the rat Interleukin-2 receptor gamma coding region with the ecto-domain of a human Interleukin-2 receptor gamma; or,

(d) at least a 3 kb deletion of the Interleukin-2 receptor gamma locus comprising the Interleukin-2 receptor gamma coding region.

57. The method of any one of claim 51-55, wherein the targeted genetic modification of the ApoE locus comprises:

(a) a deletion of the entire ApoE coding region or a portion thereof; or,

(b) at least a 1.8 kb deletion of the ApoE locus comprising the ApoE coding region.

58. The method of any one of claim 51-55, wherein the targeted genetic modification of the Rag2 locus comprises:

(a) a deletion of the entire Rag2 coding region or a portion thereof; or,

(b) at least a 5.7 kb deletion of the Rag2 locus comprising the Rag2 coding region.

59. The method of any one of claim 51-55, wherein the targeted genetic modification of the Rag1/Rag2 locus comprises:

(a) a deletion of the entire Rag2 coding region or a portion thereof and a deletion of the entire Rag1 coding region or portion thereof; or,

(b) a deletion of at least 16 kb of the Rag2/Rag1 locus comprising the Rag2 and Rag1 coding regions.

60. The method of any one of claim 51-59, wherein the insert nucleic acid comprises an expression cassette comprising a polynucleotide encoding a selective marker.

61. The method claim 60, wherein the expression cassette comprises a lacZ gene operably linked to an endogenous promoter at the genomic locus and a human ubiquitin promoter operably linked to a selective marker gene.

62. The method of any one of claims 51-60, wherein the insert nucleic acid comprises a self-deleting selection cassette.

63. The method of claim 62, wherein the self-deleting selection cassette comprises a selective marker operably linked to a promoter active in the rat pluripotent cell and a polynucleotide encoding a recombinase operably linked to a male germ cell-specific promoter, wherein the self-deleting cassette is flanked by recombination recognition sites recognized by the recombinase.

64. The method of claim 63, wherein

(a) the male germ cell-specific promoter is a Protamine-1 promoter; or,

(b) the recombinase gene encodes Cre and the recombination recognition sites are loxP sites.

65. The method of claim 53, wherein the insertion of the exogenous nucleic acid

~~sequence at the genomic locus comprises a reporter nucleic acid sequence operably linked to an endogenous Interleukin-2 receptor gamma promoter, an endogenous ApoE promoter, an endogenous Rag1 promoter, or an endogenous Rag2 promoter.~~

~~66. The method of claim 65, wherein the reporter nucleic acid sequence encodes a reporter comprising β-galactosidase , mPlum, mCherry, tdTomato, mStrawberry, J-Red, DsRed, mOrange, mKO, mCitrine, Venus, YPet, enhanced yellow fluorescent protein (EYFP), Emerald, enhanced green fluorescent protein (EGFP), CyPet, cyan fluorescent protein (CFP), Cerulean, T-Sapphire, luciferase, alkaline phosphatase, or a combination thereof.~~

~~67. The method of any one of claim 51-66, wherein the pluripotent rat cell is a rat embryonic stem (ES) cell.~~

~~68. The method of any one of claim 51-67, wherein the pluripotent rat cell~~

~~(a) is derived from a DA strain or an ACI strain; or,~~

~~(b) is characterized by expression of a pluripotency marker comprising Oct-4, Sox-2, alkaline phosphatase, or a combination thereof; or,~~

~~(c) is characterized by one or more of the following characteristics:~~

~~(i) lack of expression of one or more pluripotency markers comprising c-Myc, Ecat1, and/or Rexo1;~~

~~(ii) lack of expression of mesodermal markers comprising Brachyury and/or Bmpr2;~~

~~(iii) lack of expression of one or more endodermal markers comprising Gata6, Sox17 and/or Sox7; or~~

~~(iv) lack of expression of one or more neural markers comprising Nestin and/or Pax6.~~

~~69. The method of any one of claim 51-68, further comprising identifying the targeted genetic modification at the target genomic locus~~1-19, wherein the identification step employs a quantitative assay for assessing a modification of allele (MOA) at the ~~target~~ genomic locus of interest.

~~70.~~ 21. The method of any one of ~~claim 51-69~~claims 1-20, wherein ~~introducing steps~~steps (a) ~~further comprises introducing a second nucleic acid encoding a nuclease agent that promotes a homologous recombination between~~and (b) are sequentially repeated to allow for the ~~targeting vector and~~targeted integration of at least two insert nucleic acids into the ~~target~~ genomic locus of interest.

22. A method for targeted modification of a genomic locus of interest in ~~the~~a pluripotent rat cell~~.~~, comprising:

~~71. The method of claim 70, wherein the nuclease agent comprises a chimeric protein comprising~~(a) targeting a ~~zinc finger-based DNA binding domain fused to a FokI endonuclease.~~

~~72. The method of claim 71, wherein the method results in bi-allelic modification of the target genomic locus.~~

~~73. The method of any one of claim 51-70, wherein introducing step (a) further comprises introducing into the pluripotent rat cell:~~

~~(i) a first expression construct comprising a first promoter operably linked to a first nucleic acid sequence encoding a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) associated (Cas) protein,~~

~~(ii) a second expression construct comprising a second promoter operably linked to a genomic target sequence linked to a guide RNA (gRNA),~~

~~wherein the genomic target sequence is immediately flanked on the 3' end by a Protospacer Adjacent Motif (PAM) sequence.~~

~~74. The method of claim 73, wherein the~~ genomic locus of interest ~~comprises the nucleotide sequence of SEQ ID NO:~~in a pluripotent rat cell according to the method of any one of claims 1~~.~~

~~75. The method of claim 73 or 74~~-21 to form a genetically modified pluripotent rat cell, wherein the ~~gRNA~~insert nucleic acid comprises a ~~third~~human nucleic acid

~~sequence encoding a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) RNA (crRNA);~~ and ~~a trans-activating CRISPR RNA (tracrRNA).~~

~~76. The method of claim 73, wherein the Cas protein is Cas9.~~

~~77. The method of claim 73, 74, or 75, wherein the gRNA comprises:~~

~~(a) the chimeric RNA of the nucleic acid sequence of SEQ ID NO: 2; or,~~

~~(b) the chimeric RNA of the nucleic acid sequence of SEQ ID NO: 3.~~

~~78. The method of claim 75, wherein the crRNA comprises SEQ ID NO: 4; SEQ ID NO: 5; or SEQ ID NO: 6.~~

~~79. The method of claim 75, wherein the tracrRNA comprises SEQ ID NO: 7 or SEQ ID NO: 8.~~

~~(~~(b) introducing the genetically modified pluripotent rat cell into a host rat embryo.