HSCs are unable to produce edited HSCs that persist long enough to produce an effective therapeutic benefit. Thus, improved gene editing techniques for use with HSCs are needed.

SUMMARY

[0007] In one aspect, provided herein is a method for genome modification at a target locus in a hematopoietic stem cell (HSC), comprising: (a) introducing a nuclease or nucleic acid encoding the nuclease into the HSC, wherein the target locus comprises a first recognition sequence for the nuclease; and (b) introducing a double-stranded donor nucleic acid into the HSC, wherein the double-stranded nucleic acid comprises an exogenous nucleic acid sequence and is configured to be inserted into the target locus by a homology-independent mechanism. In some examples, the HSC is a long-term engrafting HSC (LT-HSC) or a SCID-repopulating cell. In some examples, the HSC is characterized by the following markers: Lin^- (lineage negative)/$CD34^+$/$CD38^-$/$CD90^+$/$CD45RA^-$. In some examples, Lin^- is characterized as one or more of $CD235a^-$, $CD41a^-$, $CD3^-$, $CD19^-$, $CD14^-$, $CD16^-$, $CD20^-$, and $CD56^-$. In some examples, Lin^- is characterized as $CD235a^-$/$CD41a^-$/$CD3^-$/$CD19^-$/$CD14^-$/$CD16^-$/$CD20^-$/$CD56^-$.

[0008] In another aspect, provided herein is a method for genome modification at a target locus in a quiescent T cell, comprising: (a) introducing a nuclease or nucleic acid encoding the nuclease into the quiescent T cell, wherein the target locus comprises a first recognition sequence for the nuclease; and (b) introducing a double-stranded donor nucleic acid into the quiescent T cell, wherein the double-stranded nucleic acid comprises an exogenous nucleic acid sequence and is configured to be inserted into the target locus by a homology-independent mechanism. In some examples, the quiescent T cell is a non-activated T cell.

[0009] In another aspect, provided herein is a method for genome modification at a target locus in an HDR-deficient cell, comprising: (a) introducing a nuclease or nucleic acid encoding the nuclease into the HDR-deficient cell, wherein the target locus comprises a first recognition sequence for the nuclease; (b) introducing a double-stranded donor nucleic acid into the HDR-deficient cell, wherein the double-stranded nucleic acid comprises an exogenous nucleic acid sequence and is configured to be inserted into the target locus by a homology-independent mechanism, and (c) culturing the HDR-deficient cell for a time sufficient for integration of the double-stranded donor nucleic acid into the target locus, wherein steps (a), (b), and (c) are carried out such that the insertion efficiency for the double-stranded donor nucleic acid is at least

about 4%. In some examples, the insertion efficiency for the double-stranded donor nucleic acid is at least about 8%.

[0010] In some examples, according to any of the methods for genome modification described above, the double-stranded donor nucleic acid further comprises a second recognition sequence for the nuclease flanking a first end of the exogenous nucleic acid sequence. In some examples, the double-stranded donor nucleic acid further comprises a third recognition sequence flanking a second end of the exogenous nucleic acid sequence. In some examples, the double-stranded donor nucleic acid is cleaved at the second and/or third recognition sequence following introduction into the cell.

[0011] In some examples, according to any of the methods for genome modification described above, the double-stranded donor nucleic acid is configured such that insertion of the cleaved double-stranded donor nucleic acid into the target locus in a desired orientation does not create recognition sequences for the nuclease in the modified target locus and insertion of the cleaved double-stranded donor nucleic acid into the target locus in the other orientation creates a recognition sequence for the nuclease in the modified target locus.

[0012] In some examples, according to any of the methods for genome modification described above, the nuclease is an RNA-guided endonuclease (RGEN), each of the recognition sequences for the nuclease in the target locus and double-stranded donor nucleic acid is a protospacer sequence, and the method further comprises introducing into the cell one or more gRNAs targeting one or more of the protospacer sequences. In some examples, the method comprises introducing into the cell a gRNA comprising a spacer targeting the protospacers in the target locus and the donor nucleic acid. In some examples, the protospacer in the target locus is in a forward orientation, the exogenous nucleic acid in the double-stranded donor nucleic acid is in a forward orientation, and the protospacers in the double-stranded donor nucleic acid are in a reverse orientation. In some examples, the protospacers in the target locus and the donor nucleic acid are the same. In some examples, at least one of the protospacers in the target locus and the donor nucleic acid is a delayed-action protospacer (DAP) incompletely matching the gRNA spacer. In some examples, the DAP i) is shorter in length than the gRNA spacer by at least about 1 nucleotide; and/or ii) comprises at least about 1 nucleotide mismatch with the gRNA spacer. In some examples, the protospacers in the donor nucleic acid are DAPs, and the protospacer in the target locus completely matches the gRNA spacer. In some examples, the double-stranded donor

nucleic acid comprises two DAPs flanking the exogenous nucleic acid sequence. In some examples, the protospacers in the donor nucleic acid completely match the gRNA spacer, and the protospacer in the target locus is a DAP.

[0013] In some examples, according to any of the methods for genome modification described above employing an RGEN, the RGEN is a Cas9 nuclease.

[0014] In some examples, according to any of the methods for genome modification described above employing an RGEN, the method comprises introducing into the cell a ribonucleoprotein (RNP) comprising the RGEN and the one or more gRNAs.

[0015] In some examples, according to any of the methods for genome modification described above employing an RGEN, the method comprises introducing into the cell an mRNA encoding the RGEN.

[0016] In some examples, according to any of the methods for genome modification described above, the double-stranded donor nucleic acid is a double-stranded virus genome. In some examples, the double-stranded virus genome is an adenovirus genome, a lentivirus genome, or an adeno-associated virus (AAV) genome. In some examples, the AAV genome is a self-complementary AAV (scAAV) genome. In some examples, the scAAV genome is an scAAV6 genome.

[0017] In some examples, according to any of the methods for genome modification described above, the nuclease or nucleic acid encoding the nuclease is introduced into the cell before the donor nucleic acid is introduced into the cell. In some examples, the nuclease or nucleic acid encoding the nuclease is introduced into the cell no more than 1 hour before the donor nucleic acid is introduced into the cell. In some examples, the nuclease or nucleic acid encoding the nuclease is introduced into the cell no more than 5 minutes before the donor nucleic acid is introduced into the cell.

[0018] In some examples, according to any of the methods for genome modification described above, the cell is cultured under hypoxic conditions.

[0019] In some examples, according to any of the methods for genome modification described above, the cell is cultured no longer than about 48 hours prior to introducing the nuclease or nucleic acid encoding the nuclease and the donor nucleic acid into the cell. In some examples, the cell is cultured no longer than about 24 hours prior to introducing the nuclease or nucleic acid encoding the nuclease and the donor nucleic acid into the cell. In some examples, the cell is

cultured no longer than about 2 hours prior to introducing the nuclease or nucleic acid encoding the nuclease and the donor nucleic acid into the cell.

[0020] In some examples, according to any of the methods for genome modification described above, the cell is cultured in the presence of a Notch ligand. In some examples, the Notch ligand is a Delta-like Notch ligand (DLL), Jagged-1, Jagged-2, or a conjugate thereof. In some examples, the Delta-like Notch ligand is DLL1, DLL3, or DLL4. In some examples, the Notch ligand is Fc-DLL1, Fc-DLL3, Fc-DLL4, Fc-Jagged-1, or Fc-Jagged-2.

[0021] In a further aspect, the present disclosure provides the use of edited HSCs comprising an exogenous nucleic acid sequence inserted at a target locus in the manufacture of a medicament for engraftment in an individual, comprising that: (a) the method as described herein is carried out on an input population of HSCs obtained from the individual to generate an output population of HSCs comprising a population of edited HSCs comprising the exogenous nucleic acid sequence inserted at the target locus; and (b) the population of edited HSCs is to be administered to the individual such that the edited HSCs are to be engrafted in the individual In another example, provided herein is a method for engraftment in an individual of edited HSCs comprising an exogenous nucleic acid sequence inserted at a target locus, comprising: (a) carrying out a method for genome modification according to any of the examples described above on an input population of HSCs obtained from the individual to generate an output population of HSCs comprising a population of edited HSCs comprising the exogenous nucleic acid sequence inserted at the target locus; and (b) administering the population of edited HSCs to the individual such that the edited HSCs are engrafted in the individual (e.g., such that the edited HSCs persist in the individual for at least about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or more weeks). In some examples, the amount of engraftment of edited HSC in the individual is the same or greater than the amount of engraftment of corresponding edited HSCs prepared using a homology-dependent mechanism. In some examples, the input population of HSCs obtained from the individual comprises a mixed population of HSCs comprising LT-HSCs and short-term engrafting HSCs (ST-HSCs), and the population of edited HSCs that engrafted comprise edited LT-HSCs. In some examples, administering the population of edited HSCs to the individual comprises administering the output population of HSCs to the individual.

[0022] In another aspect, provided herein is an engineered HSC comprising: (a) a target locus comprising a first recognition sequence for a nuclease; (b) the nuclease or a nucleic acid

encoding the nuclease; and (c) a double-stranded donor nucleic acid comprising an exogenous nucleic acid sequence, wherein the double-stranded donor nucleic acid is configured to be inserted into the target locus by a homology-independent mechanism. In some examples, the HSC is an LT-HSC or a SCID-repopulating cell. In some examples, the HSC is characterized by the following markers: $Lin^-/CD34^+/CD38^-/CD90^+/CD45RA^-$. In some examples, Lin^- is characterized as one or more of $CD235a^-$, $CD41a^-$, $CD3^-$, $CD19^-$, $CD14^-$, $CD16^-$, $CD20^-$, and $CD56^-$. In some examples, Lin^- is characterized as $CD235a^-/CD41a^-/CD3^-/CD19^-/CD14^-/CD16^-/CD20^-/CD56^-$.

[0023] In another aspect, provided herein is an engineered HSC prepared by a method for genome modification at a target locus in a cell according to any of the examples described above. In some examples, the method comprises: (a) introducing a nuclease or nucleic acid encoding the nuclease into an input HSC, wherein the target locus comprises a first recognition sequence for the nuclease; and (b) introducing a double-stranded donor nucleic acid into the input HSC, wherein the double-stranded nucleic acid comprises an exogenous nucleic acid sequence and is configured to be inserted into the target locus by a homology-independent mechanism (e.g., NHEJ). In some examples, the HSC is an LT-HSC or a SCID-repopulating cell. In some examples, the HSC is characterized by the following markers: $Lin^-/CD34^+/CD38^-/CD90^+/CD45RA^-$. In some examples, Lin^- is characterized as one or more of $CD235a^-$, $CD41a^-$, $CD3^-$, $CD19^-$, $CD14^-$, $CD16^-$, $CD20^-$, and $CD56^-$. In some examples, Lin^- is characterized as $CD235a^-/CD41a^-/CD3^-/CD19^-/CD14^-/CD16^-/CD20^-/CD56^-$.

[0024] In another example, provided herein is an engineered quiescent T cell comprising: (a) a target locus comprising a first recognition sequence for a nuclease; (b) the nuclease or a nucleic acid encoding the nuclease; and (c) a double-stranded donor nucleic acid comprising an exogenous nucleic acid sequence, wherein the double-stranded donor nucleic acid is configured to be inserted into the target locus by a homology-independent mechanism. In some examples, the quiescent T cell is a non-activated T cell.

[0025] In another aspect, provided herein is an engineered quiescent T cell prepared by a method for genome modification at a target locus in a cell according to any of the examples described above. In some examples, the method comprises: (a) introducing a nuclease or nucleic acid encoding the nuclease into an input quiescent T cell, wherein the target locus comprises a first recognition sequence for the nuclease; and (b) introducing a double-stranded donor nucleic

acid into the input quiescent T cell, wherein the double-stranded nucleic acid comprises an exogenous nucleic acid sequence and is configured to be inserted into the target locus by a homology-independent mechanism (e.g., NHEJ). In some examples, the quiescent T cell is a non-activated T cell.

[0026] In some examples, according to any of the engineered cells described above, the double-stranded donor nucleic acid further comprises a second recognition sequence for the nuclease flanking a first end of the exogenous nucleic acid sequence. In some examples, the double-stranded donor nucleic acid further comprises a third recognition sequence flanking a second end of the exogenous nucleic acid sequence.

[0027] In some examples, according to any of the engineered cells described above, the double-stranded donor nucleic acid is configured such that insertion of the cleaved double-stranded donor nucleic acid into the target locus in a desired orientation does not create recognition sequences for the nuclease in the modified target locus and insertion of the cleaved double-stranded donor nucleic acid into the target locus in the other orientation creates a recognition sequence for the nuclease in the modified target locus.

[0028] In some examples, according to any of the engineered cells described above, the nuclease is an RNA-guided endonuclease (RGEN), each of the recognition sequences for the nuclease in the target locus and double-stranded donor nucleic acid is a protospacer sequence, and the engineered cell further comprises one or more gRNAs specific for each distinct recognition sequence for the nuclease in the target locus and double-stranded donor nucleic acid. In some examples, the engineered cell comprises a gRNA comprising a spacer targeting the protospacers in the target locus and the donor nucleic acid. In some examples, the protospacer in the target locus is in a forward orientation, the exogenous nucleic acid in the double-stranded donor nucleic acid is in a forward orientation, and the protospacers in the double-stranded donor nucleic acid are in a reverse orientation. In some examples, the protospacers in the target locus and the donor nucleic acid are the same. In some examples, at least one of the protospacers in the target locus and the donor nucleic acid is a delayed-action protospacer (DAP) incompletely matching the gRNA spacer. In some examples, the DAP i) is shorter in length than the gRNA spacer by at least about 1 nucleotide; and/or ii) comprises at least about 1 nucleotide mismatch with the gRNA spacer. In some examples, the protospacers in the donor nucleic acid are DAPs, and the protospacer in the target locus completely matches the gRNA spacer. In some examples,

the double-stranded donor nucleic acid comprises two DAPs flanking the exogenous nucleic acid sequence. In some examples, the protospacers in the donor nucleic acid completely match the gRNA spacer, and the protospacer in the target locus is a DAP.

[0029] In some examples, according to any of the engineered cells comprising an RGEN described above, the RGEN is a Cas9 nuclease.

[0030] In some examples, according to any of the engineered cells comprising an RGEN described above, the engineered cell comprises a ribonucleoprotein (RNP) comprising the RGEN and the one or more gRNAs.

[0031] In some examples, according to any of the engineered cells comprising an RGEN described above, the engineered cell comprises an mRNA encoding the RGEN.

[0032] In some examples, according to any of the engineered cells described above, the double-stranded donor nucleic acid is a double-stranded virus genome. In some examples, the double-stranded virus genome is an adenovirus genome, a lentivirus genome, or an adeno-associated virus (AAV) genome. In some examples, the AAV genome is a self-complementary AAV (scAAV) genome. In some examples, the scAAV genome is an scAAV6 genome.

[0033] In a further example, the present disclosure provides the use of an engineered cell as described herein in the manufacture of a medicament for treating a disease or condition in a subject, wherein the disease or condition is characterized by deficient expression of a functional protein, wherein the exogenous nucleic acid encodes a functional form of the protein that can be expressed in the engineered cell. In another example, provided herein is a method of treating a disease or condition in a subject, wherein the disease or condition is characterized by deficient expression of a functional protein, comprising administering to the subject an engineered cell according to any of the examples described above, wherein the exogenous nucleic acid encodes a functional form of the protein that can be expressed in the engineered cell. In some examples, the disease or condition is SCID, and wherein the exogenous nucleic acid comprises a functional form of a gene mutated in the individual involved in lymphoid development or lymphocyte proliferation and/or metabolism. In some examples, the exogenous nucleic acid encodes a functional form of IL2Rg, RAG1, IL7R, ADA, or PNP. In some examples, the disease or

condition is Gaucher disease, Fabry disease, mucopolysaccharidosis types I-IX, or adrenoleukodystrophy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] FIG. 1A shows flow cytometry results at day 14 (D14) for NHEJ-mediated targeted integration as determined by GFP expression in K562 cells treated with pCR2.1-TOPO-psSCRAM-SA-P2A-H2Bj-Venus-BGHpA-psSCRAM (0-cut vector plasmid) or pCR2.1-TOPO-psAAVS1-SA-P2A-H2Bj-Venus-BGHpA-psSCRAM (1-cut vector plasmid) at the indicated concentrations in the presence or absence of Cas9/gRNA RNP, where pCR2.1-TOPO is the vector plasmid backbone, psSCRAM is a scrambled protospacer sequence with the PAM, psAAVS1 is the same protospacer sequence and as the genomic DNA sequence the Cas9/gRNA RNP cleaves with the PAM, SA is the splice acceptor, P2A is a peptide cleavage signal

CLAIMS

WHAT IS CLAIMED IS:

1. A method for genome modification at a target locus in a hematopoietic stem cell (HSC), comprising:

(a) introducing a nuclease or nucleic acid encoding the nuclease into the HSC, wherein the target locus comprises a first recognition sequence for the nuclease;

(b) introducing a double-stranded donor nucleic acid into the HSC, wherein the double-stranded nucleic acid comprises an exogenous nucleic acid sequence and is configured to be inserted into the target locus by a homology-independent mechanism.

2. The method of claim 1, wherein the HSC is a long-term engrafting HSC (LT-HSC) or a SCID-repopulating cell.

3. The method of claim 1, wherein the HSC is characterized by the following markers: $Lin^-/CD34^+/CD38^-/CD90^+/CD45RA^-$.

4. The method of claim 3, wherein Lin^- is characterized as one or more of $CD235a^-$, $CD41a^-$, $CD3^-$, $CD19^-$, $CD14^-$, $CD16^-$, $CD20^-$, and $CD56^-$.

5. The method of claim 4, wherein Lin^- is characterized as $CD235a^-/CD41a^-/CD3^-/CD19^-/CD14^-/CD16^-/CD20^-/CD56^-$.

6. A method for genome modification at a target locus in a quiescent T cell, comprising:

(a) introducing a nuclease or nucleic acid encoding the nuclease into the quiescent T cell, wherein the target locus comprises a first recognition sequence for the nuclease;

(b) introducing a double-stranded donor nucleic acid into the quiescent T cell, wherein the double-stranded nucleic acid comprises an exogenous nucleic acid sequence and is configured to be inserted into the target locus by a homology-independent mechanism.

7. The method of claim 6, wherein the quiescent T cell is a non-activated T cell.

8. A method for genome modification at a target locus in an HDR-deficient cell, comprising:

(a) introducing a nuclease or nucleic acid encoding the nuclease into the HDR-deficient cell, wherein the target locus comprises a first recognition sequence for the nuclease;

(b) introducing a double-stranded donor nucleic acid into the HDR-deficient cell, wherein the double-stranded nucleic acid comprises an exogenous nucleic acid sequence and is configured to be inserted into the target locus by a homology-independent mechanism, and

(c) culturing the HDR-deficient cell for a time sufficient for integration of the double-stranded donor nucleic acid into the target locus, wherein

steps (a), (b), and (c) are carried out such that the insertion efficiency for the double-stranded donor nucleic acid is at least about 4%.

9. The method of claim 8, wherein the insertion efficiency for the double-stranded donor nucleic acid is at least about 8%.

10. The method of any one of claims 1-9, wherein the double-stranded donor nucleic acid further comprises a second recognition sequence for the nuclease flanking a first end of the exogenous nucleic acid sequence.

11. The method of claim 10, wherein the double-stranded donor nucleic acid further comprises a third recognition sequence flanking a second end of the exogenous nucleic acid sequence.

12. The method of claim 10 or 11, wherein the double-stranded donor nucleic acid is cleaved at the second and/or third recognition sequence following introduction into the cell.

13. The method of any one of claims 1-12, wherein the double-stranded donor nucleic acid is configured such that insertion of the cleaved double-stranded donor nucleic acid into the target locus in a desired orientation does not create recognition sequences for the nuclease in the modified target locus and insertion of the cleaved double-stranded donor nucleic acid into the target locus in the other orientation creates a recognition sequence for the nuclease in the modified target locus.

14. The method of any one of claims 1-13, wherein the nuclease is an RNA-guided endonuclease (RGEN), each of the recognition sequences for the nuclease in the target locus and double-stranded donor nucleic acid is a protospacer sequence, and the method further comprises introducing into the cell one or more gRNAs targeting one or more of the protospacer sequences.

15. The method of claim 14, comprising introducing into the cell a gRNA comprising a spacer targeting the protospacers in the target locus and the donor nucleic acid.

16. The method of claim 15, wherein the protospacer in the target locus is in a forward orientation, the exogenous nucleic acid in the double-stranded donor nucleic acid is in a forward orientation, and the protospacers in the double-stranded donor nucleic acid are in a reverse orientation.

17. The method of claim 15 or 16, wherein the protospacers in the target locus and the donor nucleic acid are the same.

18. The method of claim 15 or 16, wherein at least one of the protospacers in the target locus and the donor nucleic acid is a delayed-action protospacer (DAP) incompletely matching the gRNA spacer.

19. The method of claim 18, wherein the DAP i) is shorter in length than the gRNA spacer by at least about 1 nucleotide; and/or ii) comprises at least about 1 nucleotide mismatch with the gRNA spacer.

20. The method of claim 18 or 19, wherein the protospacers in the donor nucleic acid are DAPs, and the protospacer in the target locus completely matches the gRNA spacer.

21. The method of claim 20, wherein the double-stranded donor nucleic acid comprises two DAPs flanking the exogenous nucleic acid sequence.

22. The method of claim 18 or 19, wherein the protospacers in the donor nucleic acid completely match the gRNA spacer, and the protospacer in the target locus is a DAP.

23. The method of any one of claims 14-22, wherein the RGEN is a Cas9 nuclease.

24. The method of any one of claims 14-23, comprising introducing into the cell a ribonucleoprotein (RNP) comprising the RGEN and the one or more gRNAs.

25. The method of any one of claims 14-23, comprising introducing into the cell an mRNA encoding the RGEN.

26. The method of any one of claims 1-25, wherein the double-stranded donor nucleic acid is a double-stranded virus genome.

27. The method of claim 26, wherein the double-stranded virus genome is an adenovirus genome, a lentivirus genome, or an adeno-associated virus (AAV) genome.

28. The method of claim 27, wherein the AAV genome is a self-complementary AAV (scAAV) genome.

29. The method of claim 28, wherein the scAAV genome is an scAAV6 genome.

30. The method of any one of claims 1-29, wherein the nuclease or nucleic acid encoding the nuclease is introduced into the cell before the donor nucleic acid is introduced into the cell.

31. The method of claim 30, wherein the nuclease or nucleic acid encoding the nuclease is introduced into the cell no more than 1 hour before the donor nucleic acid is introduced into the cell.

32. The method of claim 31, wherein the nuclease or nucleic acid encoding the nuclease is introduced into the cell no more than 5 minutes before the donor nucleic acid is introduced into the cell.

33. The method of any one of claims 1-32, wherein the cell is cultured under hypoxic conditions.

34. The method of any one of claims 1-33, wherein the cell is cultured no longer than about 48 hours prior to introducing the nuclease or nucleic acid encoding the nuclease and the donor nucleic acid into the cell.

35. The method of claim 34, wherein the cell is cultured no longer than about 24 hours prior to introducing the nuclease or nucleic acid encoding the nuclease and the donor nucleic acid into the cell.

36. The method of claim 35, wherein the cell is cultured no longer than about 2 hours prior to introducing the nuclease or nucleic acid encoding the nuclease and the donor nucleic acid into the cell.

37. The method of any one of claims 1-36, wherein the cell is cultured in the presence of a Notch ligand.

38. The method of claim 37, wherein the Notch ligand is a Delta-like Notch ligand (DLL), Jagged-1, Jagged-2, or a conjugate thereof.

39. The method of claim 38, wherein the Delta-like Notch ligand is DLL1, DLL3, or DLL4.

40. The method of claim 39, wherein the Notch ligand is Fc-DLL1, Fc-DLL3, Fc-DLL4, Fc-Jagged-1, or Fc-Jagged-2.

41. Use of edited HSCs comprising an exogenous nucleic acid sequence inserted at a target locus in the manufacture of a medicament for engraftment in an individual, comprising that:

(a) the method of any one of claims 1-40 is carried out on an input population of HSCs obtained from the individual to generate an output population of HSCs comprising a population of edited HSCs comprising the exogenous nucleic acid sequence inserted at the target locus; and

(b) the population of edited HSCs is to be administered to the individual such that the edited HSCs are to be engrafted in the individual.

42. The use of claim 41, wherein the amount of engraftment of edited HSC in the individual is the same or greater than the amount of engraftment of corresponding edited HSCs prepared using a homology-dependent mechanism.

43. The use of claim 41 or 42, wherein the input population of HSCs obtained from the individual comprises a mixed population of HSCs comprising LT-HSCs and short-term engrafting HSCs (ST-HSCs), and wherein the population of edited HSCs that engrafted comprise edited LT-HSCs.

44. The use of any one of claims 41-43, wherein the population of edited HSCs to be administered to the individual comprises the output population of HSCs to be administered to the individual.

45. An engineered HSC prepared by a method for genome modification at a target locus in an HSC according to any one of claims 1-5 and 10-40.

46. An engineered quiescent T cell prepared by a method for genome modification at a target locus in a quiescent T cell according to any one of claims 6-7 and 10-40.

47. Use of an engineered cell according to claim 45 or 46 in the manufacture of a medicament for treating a disease or condition in a subject, wherein the disease or condition is characterized by deficient expression of a functional protein, wherein the exogenous nucleic acid encodes a functional form of the protein that can be expressed in the engineered cell.

48. The use of claim 47, wherein the disease or condition is SCID, and wherein the exogenous nucleic acid comprises a functional form of a gene mutated in the individual involved in lymphoid development or lymphocyte proliferation and/or metabolism.

49. The use of claim 48, wherein the exogenous nucleic acid encodes a functional form of IL2Rg, RAG1, IL7R, ADA, or PNP.

50. The use of claim 47, wherein the disease or condition is Gaucher disease, Fabry disease, mucopolysaccharidosis types I-IX, or adrenoleukodystrophy.