WHAT IS CLAIMED IS:

1. A method of generating a population of differentiated cells comprising:

(a) contacting a first plurality of progenitor cells with a CRISPR/Cas system comprising a guide RNA (gRNA) targeting an inessential portion of a promoter of a gene;

(b) inserting biallelically by homologous recombination a construct comprising a tissue-specific promoter and at least a portion of the gene, wherein the gene is selected from the group consisting of: AACS, AADAT, AASDHPPT, AASS, ACAT1, ACCS, ACCSL, ACO1, ACO2, ACSS3, ADSL, ADSS, ADSSL1, ALAD, ALAS1, ALAS2, ALDH1A1, ALDH1A2, ALDH1A3, ALDH1B1, ALDH2, AMD1, ASL, ASS1, ATF4, ATF5, AZIN1, AZIN2, BCAT1, BCAT2, CAD, CBS, CBSL, CCBL1, CCBL2, CCS, CEBPA, CEBPB, CEBPD, CEBPE, CEBPG, CH25H, COQ6, CPS1, CTH, CYP51A1, DECR1, DHFR, DHFRL1, DHODH, DHRS7, DHRS7B, DHRS7C, DPYD, DUT, ETFDH, FAXDC2, FDFT1, FDPS, FDXR, FH, FPGS, G6PD, GCAT, GCH1, GCLC, GFPT1, GFPT2, GLRX5, GLUL, GMPS, GPT, GPT2, GSX2, H6PD, HAAO, HLCS, HMBS, HMGCL, HMGCLL1, HMGCS1, HMGCS2, HOXA1, HOXA10, HOXA11, HOXA13, HOXA2, HOXA3, HOXA4, HOXA5, HOXA6, HOXA7, HOXA9, HOXB1, HOXB13, HOXB2, HOXB3, HOXB4, HOXB5, HOXB6, HOXB7, HOXB8, HOXB9, HOXC10, HOXC11, HOXC12, HOXC13, HOXC4, HOXC5, HOXC6, HOXC8, HOXC9, HOXD1, HOXD10, HOXD11, HOXD12, HOXD13, HOXD3, HOXD4, HOXD8, HOXD9, HRSP12, HSD11B1, HSD11B1L, HSD17B12, HSD17B3, HSD17B7, HSD17B7P2, HSDL1, HSDL2, IBA57, IDO1, IDO2, IL4I1, ILVBL, IP6K1, IP6K2, IP6K3, IPMK, IREB2, ISCA1, ISCA1P1, ISCA2, KATNA1, KATNAL1, KATNAL2, KDM1B, KDSR, KMO, KYNU, LGSN, LSS, MARS, MARS2, MAX, MITF, MLX, MMS19, MPC1, MPC1L, MPI, MSMO1, MTHFD1, MTHFD1L, MTHFD2, MTHFD2L, MTHFR, MTRR, MVK, MYB, MYBL1, MYBL2, NAGS, ODC1, OTC, PAICS, PAOX, PAPSS1, PAPSS2, PDHB, PDX1, PFAS, PIN1, PLCB1, PLCB2, PLCB3, PLCB4, PLCD1, PLCD3, PLCD4, PLCE1, PLCG1, PLCG2, PLCH1, PLCH2, PLCL1, PLCL2, PLCZ1, PM20D1, PPAT, PSAT1, PSPH, PYCR1, PYCR2, , QPRT, RDH8, RPUSD2, SCD, SCD5, SLC25A19, SLC25A26, SLC25A34, SLC25A35, SLC7A10, SLC7A11, SLC7A13, SLC7A5, SLC7A6, SLC7A7, SLC7A8, SLC7A9, SMOX, SMS, SNAPC4, SOD1, SOD3, SQLE, SRM, TAT, TFE3, TFEB, TFEC, THNSL1, THNSL2, TKT, TKTL1, TKTL2, UMPS, UROD, UROS, USF1, USF2, VPS33A, VPS33B, VPS36, VPS4A, and

VPS4B, resulting in a second plurality of progenitor cells, which isauxotrophic for an auxotrophic factor;

(c) contacting the second plurality of progenitor cells with the auxotrophic factor;

(d) stimulating differentiation of the second plurality of progenitor cells into a tissue associated with the tissue-specific promoter, wherein the gene is expressed in response to differentiation; and

(e) removing the auxotrophic factor, thereby selecting for differentiated cells to generate the population of differentiated cells.

2. The method of claim 1, further comprising contacting the plurality of progenitor cells with 5-FOA.

3. The method of claims 1 or 2, wherein the gene is a UMPS gene.

4. The method of claim 3, wherein the tissue-specific promoter replaces the promoter of the UMPS gene.

5. The method of any one of claims 1-4, wherein the auxotrophic factor is uracil or a source of uracil.

6. The method of any one of claims 1-5, wherein the construct further comprises a nucleotide sequence encoding a therapeutic factor which is expressed in response to differentiation.

7. The method of claim 6, further comprising expressing the therapeutic factor as a cassette with the at least a portion of the UMPS gene.

8. The method of any one of claims 1-7, wherein the construct is polycistronic.

9. The method of claim 8, wherein the construct comprises an internal ribosome entry site (IRES) or a peptide 2A sequence (P2A).

10. The method of any one of claims 3-9, wherein the at least a portion of the UMPS gene is a homology arm.

11. The method of any one of claims 1-10, wherein the plurality of progenitor cells is selected from the group consisting of hematopoietic stem cells (HSCs), embryonic stem cells, transdifferentiated stem cells, neural progenitor cells, mesenchymal stem cells, osteoblasts, cardiomyocytes, and combinations thereof.

12. The method of any one of claims 1-11, wherein the tissue is selected from the group consisting of adipose tissue, adrenal gland, ascites, bladder, blood, bone, bone marrow, brain, cervix, connective tissue, ear, embryonic tissue, esophagus, eye, heart, hematopoietic tissue, intestine, kidney, larynx, liver, lung, lymph, lymph node, mammary gland, mouth, muscle, nerve, ovary, pancreas, parathyroid, pharynx, pituitary gland, placenta, prostate, salivary gland, skin, spleen, stomach, testis, thymus, thyroid, tonsil, trachea, umbilical cord, uterus, endocrine, neuronal tissue, and vascular tissue.

13. The method of any one of claims 1-12, wherein the the population of differentiated cells comprises immune cells.

14. The method of claim 13, wherein the immune cells are selected from the group consisting of T cells, B cells, natural killer (NK) cells, and combinations thereof.

15. The method of any one of claims 1-14, wherein the tissue-specific promoter is selected from the group consisting of WAS proximal promoter; CD4 mini-promoter/enhancer; CD2 locus control region; CD4 minimal promoter and proximal enhancer and silencer; CD4 mini-promoter/enhancer; GATA-1 enhancer HS2 within the LTR; Ankyrin-1 and α-spectrin promoters combined or not with HS-40, GATA-1, ARE and intron 8 enhancers; Ankyrin-1 promoter/β-globin HS-40 enhancer; GATA-1 enhancer HS1 to HS2 within the retroviral LTR; Hybrid cytomegalovirus (CMV) enhancer/β-actin promoter; MCH II-specific HLA-DR promoter; Fascin promoter (pFascin); Dectin-2 gene promoter; 5′ untranslated region from the DC-STAMP; Heavy chain intronic enhancer (Eμ) and matrix attachment regions; CD19 promoter; Hybrid immunoglobulin promoter (Igk promoter, intronic Enhancer and 3′ enhancer from Ig genes); CD68L promoter and first intron; Glycoprotein Ibα promoter; Apolipoprotein E (Apo E) enhancer/alpha1-antitrypsin (hAAT) promoter (ApoE/hAAT); HAAT promoter/Apo E locus control region; Albumin promoter; HAAT promoter/four copies of the Apo E enhancer; Albumin and hAAT promoters/α1-microglobulin and prothrombin enhancers; HAAT

promoter/Apo E locus control region; hAAT promoter/four copies of the Apo E enhancer; TBG promoter (thyroid hormone-binding globulin promoter and α1-microglobulin/bikunin enhancer); DC172 promoter (α1-antitrypsin promoter and α1-microglobulin enhancer); LCAT, kLSP-IVS, ApoE/hAAT and liver-fatty acid-binding protein promoters; RU486-responsive promoter; Creatine kinase promoter; Creatine kinase promoter; Synthetic muscle-specific promoter C5-12; Creatine kinase promoter; Hybrid enhancer/promoter regions of α-myosin and creatine kinase (MHCK7); Hybrid enhancer/promoter regions of α-myosin and creatine kinase; Synthetic muscle-specific promoter C5-12; Cardiac troponin-I proximal promoter; E-selectin and KDR promoters; Prepro-endothelin-1 promoter; KDR promoter/hypoxia-responsive element; Flt-1 promoter; Flt-1 promoter; ICAM-2 promoter; Synthetic endothelial promoter; Endothelin-1 gene promoter; Amylase promoter; Insulin and human pdx-1 promoters; TRE-regulated insulin promoter; Enolase promoter; Enolase promoter; TRE-regulated synapsin promoter; Synapsin 1 promoter; PDGF-β promoter/CMV enhancer; PDGF-β, synapsin, tubulin-α and Ca2+/calmodulin-PK2 promoters combined with CMV enhancer; Phosphate-activated glutaminase and vesicular glutamate transporter-1 promoters; Glutamic acid decarboxylase-67 promoter; Tyrosine hydroxylase promoter; Neurofilament heavy gene promoter; Human red opsin promoter; Keratin-18 promoter; keratin-14 (K14) promoter; and Keratin-5 promoter.

16. The method of any one of claims 1-15, wherein the construct is tagged with a conditional destabilization domain or a conditional ribozyme switch.

17. A method of generating a population of differentiated cells comprising:

(a) contacting a first plurality of progenitor cells with a DNA sequence encoding one or more progenitor cell-specific miRNA target sites, wherein the DNA sequence is knocked into an auxotrophy-inducing gene resulting in a second plurality of progenitor cells, which is auxotrophic for an auxotrophic factor, and wherein a progenitor cell-specific miRNA that binds the miRNA target sites is expressed in the second plurality of progenitor cells;

(b) contacting the second plurality of progenitor cells with the auxotrophic factor;

(c) stimulating differentiation of the progenitor cells, wherein differentiation suppresses expression of the progenitor cell-specific miRNA and activates expression of the gene; and

(d) removing the auxotrophic factor, thereby selecting for differentiated cells to generate the population of differentiated cells.

18. The method of claim 17, further comprising contacting the plurality of progenitor cells with 5-FOA.

19. The method of claim 17 or 18, wherein the auxotrophy-inducing gene is selected from the group consisting of: AACS, AADAT, AASDHPPT, AASS, ACAT1, ACCS, ACCSL, ACO1, ACO2, ACSS3, ADSL, ADSS, ADSSL1, ALAD, ALAS1, ALAS2, ALDH1A1, ALDH1A2, ALDH1A3, ALDH1B1, ALDH2, AMD1, ASL, ASS1, ATF4, ATF5, AZIN1, AZIN2, BCAT1, BCAT2, CAD, CBS, CBSL, CCBL1, CCBL2, CCS, CEBPA, CEBPB, CEBPD, CEBPE, CEBPG, CH25H, COQ6, CPS1, CTH, CYP51A1, DECR1, DHFR, DHFRL1, DHODH, DHRS7, DHRS7B, DHRS7C, DPYD, DUT, ETFDH, FAXDC2, FDFT1, FDPS, FDXR, FH, FPGS, G6PD, GCAT, GCH1, GCLC, GFPT1, GFPT2, GLRX5, GLUL, GMPS, GPT, GPT2, GSX2, H6PD, HAAO, HLCS, HMBS, HMGCL, HMGCLL1, HMGCS1, HMGCS2, HOXA1, HOXA10, HOXA11, HOXA13, HOXA2, HOXA3, HOXA4, HOXA5, HOXA6, HOXA7, HOXA9, HOXB1, HOXB13, HOXB2, HOXB3, HOXB4, HOXB5, HOXB6, HOXB7, HOXB8, HOXB9, HOXC10, HOXC11, HOXC12, HOXC13, HOXC4, HOXC5, HOXC6, HOXC8, HOXC9, HOXD1, HOXD10, HOXD11, HOXD12, HOXD13, HOXD3, HOXD4, HOXD8, HOXD9, HRSP12, HSD11B1, HSD11B1L, HSD17B12, HSD17B3, HSD17B7, HSD17B7P2, HSDL1, HSDL2, IBA57, IDO1, IDO2, IL4I1, ILVBL, IP6K1, IP6K2, IP6K3, IPMK, IREB2, ISCA1, ISCA1P1, ISCA2, KATNA1, KATNAL1, KATNAL2, KDM1B, KDSR, KMO, KYNU, LGSN, LSS, MARS, MARS2, MAX, MITF, MLX, MMS19, MPC1, MPC1L, MPI, MSMO1, MTHFD1, MTHFD1L, MTHFD2, MTHFD2L, MTHFR, MTRR, MVK, MYB, MYBL1, MYBL2, NAGS, ODC1, OTC, PAICS, PAOX, PAPSS1, PAPSS2, PDHB, PDX1, PFAS, PIN1, PLCB1, PLCB2, PLCB3, PLCB4, PLCD1, PLCD3, PLCD4, PLCE1, PLCG1, PLCG2, PLCH1, PLCH2, PLCL1, PLCL2, PLCZ1, PM20D1, PPAT, PSAT1, PSPH, PYCR1, PYCR2, , QPRT, RDH8, RPUSD2, SCD, SCD5, SLC25A19, SLC25A26, SLC25A34, SLC25A35, SLC7A10, SLC7A11, SLC7A13, SLC7A5, SLC7A6, SLC7A7, SLC7A8, SLC7A9, SMOX, SMS, SNAPC4, SOD1, SOD3, SQLE, SRM, TAT, TFE3, TFEB, TFEC, THNSL1, THNSL2, TKT, TKTL1, TKTL2, UMPS, UROD, UROS, USF1, USF2, VPS33A, VPS33B, VPS36, VPS4A, and VPS4B.

20. The method of any one of claims 17-19, wherein the auxotrophy-inducing gene is uridine monophosphate synthetase (UMPS) and the one or more progenitor cell-specific miRNA target sites is present in a mRNA transcript transcribed from the UMPS gene.

21. The method of any one of claims 17-20, wherein the one or more progenitor cell-specific miRNA target sites is in the 3' untranslated region (UTR) of a transcript transcribed from the auxotrophy-inducing gene.

22. The method of any one of claims 17-21, wherein the auxotrophic factor is uracil or a source of uracil.

23. The method of any one of claims 17-22, further comprising inserting into the genome of the progenitor cells a construct comprising a gene encoding a therapeutic factor, wherein expression of the therapeutic factor is controlled by the same promoter as the promoter controlling expression of the auxotrophy-inducing gene and the differentiated cells express the therapeutic factor.

24. The method of any one of claims 17-23, further comprising expressing the therapeutic factor as a cassette in-frame with the auxotrophy-inducing gene.

25. The method of claim 24, wherein the cassette comprises an internal ribosome entry site (IRES) or a peptide 2A sequence (P2A).

26. The method of any one of claims 20-25, wherein the DNA sequence encoding the one or more progenitor cell-specific miRNA target sites further comprises a homology arm targeting the auxotrophy-inducing gene.

27. The method of any one of claims 17-26, wherein the plurality of progenitor cells is selected from the group consisting of hematopoietic stem cells (HSCs), embryonic stem cells, transdifferentiated stem cells, neural progenitor cells, mesenchymal stem cells, osteoblasts, cardiomyocytes, and combinations thereof.

28. The method of any one of claims 17-27, wherein the stimulating differentiation of the progenitor cells produces differentiated cells of a cell or tissue type selected from the group consisting of adipose tissue, adrenal gland, ascites, bladder, blood, bone, bone marrow, brain,

cervix, connective tissue, ear, embryonic tissue, esophagus, eye, heart, hematopoietic tissue, intestine, kidney, larynx, liver, lung, lymph, lymph node, mammary gland, mouth, muscle, nerve, ovary, pancreas, parathyroid, pharynx, pituitary gland, placenta, prostate, salivary gland, skin, spleen, stomach, testis, thymus, thyroid, tonsil, trachea, umbilical cord, uterus, endocrine, neuronal tissue, and vascular tissue.

29. The method of any one of claims 17-28, wherein the the population of differentiated cells comprises immune cells.

30. The method of claim 29, wherein the immune cells are selected from the group consisting of T cells, B cells, natural killer (NK) cells, and combinations thereof.

31. The method of any one of claims 17-30, wherein the gene is tagged with a conditional destabilization domain or a conditional ribozyme switch.

32. A method of alleviating auxotrophy by producing an auxotrophic factor upon differentiation, the method comprising:

(a) providing a plurality of auxotrophic progenitor cells which have been generated by knockout of an auxotrophy-inducing gene; and

(b) inserting a construct comprising an open reading frame of the auxotrophy-inducing gene into a tissue-specific gene locus, wherein expression of the tissue-specific gene is not disrupted, thereby producing the auxotrophic factor upon differentiation of the progenitor cells into the tissue associated with the tissue-specific gene locus.

33. A method of generating a population of differentiated cells comprising:

(a) contacting a first plurality of progenitor cells with a CRISPR/Cas system comprising a guide RNA (gRNA) targeting biallelically a portion of an auxotrophy-inducing gene, the auxotrophy-inducing gene comprising at least a first and a second independent functional domain, resulting in a second plurality of progenitor cells, which is auxotrophic for an auxotrophic factor;

(b) contacting the second plurality of progenitor cells with a first homologous recombination construct and a second homologous recombination construct to produce a third

plurality of progenitor cells, the first homologous recombination construct comprising a first tissue-specific promoter and at least a portion of the first independent functional domain of the auxotrophy-inducing gene, and the second homologous recombination construct comprising a second tissue-specific promoter and at least a portion of the second independent functional domain of the auxotrophy-inducing gene;

(c) contacting the third plurality of progenitor cells with the auxotrophic factor;

(d) stimulating differentiation of the progenitor cells into a cell type or tissue expressing the first and the second tissue-specific promoters, wherein the first and the second homologous recombination constructs are expressed in differentiated cells; and

(e) selecting for differentiated cells by removing the auxotrophic factor, thereby generating the population of differentiated cells.

34. The method of claim 33, wherein the auxotrophy-inducing gene further comprises:

a third independent functional domain; a third and a fourth independent functional domain; or a third, a fourth, and a fifth independent functional domain;

the method further comprising contacting the plurality of progenitor cells with, respectively: a third homologous recombination construct comprising a third tissue-specific promoter and at least a portion of the third independent functional domain; a third and a fourth homologous recombination construct comprising a third and a fourth tissue-specific promoter and at least a portion of the third and the fourth independent functional domain; or a third, a fourth, and a fifth homologous recombination construct comprising a third, a fourth, and a fifth tissue-specific promoter and at least a portion of the third, the fourth, and the fifth independent functional domain;

wherein the cell type or tissue expresses the third, the third and the fourth, or the third, the fourth, and fifth tissue-specific promoters, respectively, and the third, the third and the fourth, or the third, the fourth, and the fifth homologous recombination constructs are all expressed in differentiated cells.

35. The method of claim 33, wherein the auxotrophy-inducing gene is uridine monophosphate synthase (UMPS), the first independent functional domain comprises orotate

phosphoribosyltransferase (OPRT), and the second independent functional domain comprises orotidine 5'-phosphate decarboxylase (ODC).

36. The method of claim 34, wherein the auxotrophy-inducing gene is carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD), the first independent functional domain comprises carbamoyl-phosphate synthetase 2, the second independent functional domain comprises aspartate transcarbamylase, and the third independent functional domain comprises dihydroorotase.

37. The method of any one of claims 33-36, further comprising contacting the progenitor cells with 5-FOA.

38. The method of any one of claims 33-37, wherein one or more of the homologous recombination constructs is inserted into a safe harbor locus.

39. The method of claim 38, wherein the safe harbor locus is CCR5.

40. The method of any one of claims 33-39, wherein the auxotrophic factor is uridine.

41. The method of any one of claims 33-40, wherein one or more of the homologous recombination constructs further comprises a nucleotide sequence encoding a therapeutic factor.

42. The method of any one of claims 33-41, wherein one or more of the homologous recombination constructs is polycistronic.

43. The method of claim 42, wherein one or more of the polycistronic constructs comprises an internal ribosome entry site (IRES) or a peptide 2A sequence (P2A).

44. The method of any one of claims 33-43, wherein the plurality of progenitor cells is selected from the group consisting of hematopoietic stem cells (HSCs), embryonic stem cells, transdifferentiated stem cells, neural progenitor cells, mesenchymal stem cells, osteoblasts, cardiomyocytes, and combinations thereof.

45. The method of any one of claims 33-44, wherein the cell type or tissue is selected from the group consisting of adipose tissue, adrenal gland, ascites, bladder, blood, bone, bone

marrow, brain, cervix, connective tissue, ear, embryonic tissue, esophagus, eye, heart, hematopoietic tissue, intestine, kidney, larynx, liver, lung, lymph, lymph node, mammary gland, mouth, muscle, nerve, ovary, pancreas, parathyroid, pharynx, pituitary gland, placenta, prostate, salivary gland, skin, spleen, stomach, testis, thymus, thyroid, tonsil, trachea, umbilical cord, uterus, endocrine, neuronal, and vascular.

46. The method of any one of claims 33-45, wherein the differentiated cells are immune cells.

47. The method of claim 46, wherein the immune cells are selected from the group consisting of T cells, B cells, natural killer (NK) cells, and combinations thereof.

48. The method of any one of claims 33-47, wherein the two or more tissue-specific promoters are selected from the group consisting of WAS proximal promoter; CD4 mini-promoter/enhancer; CD2 locus control region; CD4 minimal promoter and proximal enhancer and silencer; CD4 mini-promoter/enhancer; GATA-1 enhancer HS2 within the LTR; Ankyrin-1 and α-spectrin promoters combined or not with HS-40, GATA-1, ARE and intron 8 enhancers; Ankyrin-1 promoter/β-globin HS-40 enhancer; GATA-1 enhancer HS1 to HS2 within the retroviral LTR; Hybrid cytomegalovirus (CMV) enhancer/β-actin promoter; MCH II-specific HLA-DR promoter; Fascin promoter (pFascin); Dectin-2 gene promoter; 5′ untranslated region from the DC-STAMP; Heavy chain intronic enhancer (Eμ) and matrix attachment regions; CD19 promoter; Hybrid immunoglobulin promoter (Igk promoter, intronic Enhancer and 3′ enhancer from Ig genes); CD68L promoter and first intron; Glycoprotein Ibα promoter; Apolipoprotein E (Apo E) enhancer/alpha1-antitrypsin (hAAT) promoter (ApoE/hAAT); HAAT promoter/Apo E locus control region; Albumin promoter; HAAT promoter/four copies of the Apo E enhancer; Albumin and hAAT promoters/α1-microglobulin and prothrombin enhancers; HAAT promoter/Apo E locus control region; hAAT promoter/four copies of the Apo E enhancer; TBG promoter (thyroid hormone-binding globulin promoter and α1-microglobulin/bikunin enhancer); DC172 promoter (α1-antitrypsin promoter and α1-microglobulin enhancer); LCAT, kLSP-IVS, ApoE/hAAT and liver-fatty acid-binding protein promoters; RU486-responsive promoter; Creatine kinase promoter; Creatine kinase promoter; Synthetic muscle-specific promoter C5-12; Creatine kinase promoter; Hybrid enhancer/promoter regions of α-myosin and creatine kinase

(MHCK7); Hybrid enhancer/promoter regions of α-myosin and creatine kinase; Synthetic muscle-specific promoter C5-12; Cardiac troponin-I proximal promoter; E-selectin and KDR promoters; Prepro-endothelin-1 promoter; KDR promoter/hypoxia-responsive element; Flt-1 promoter; Flt-1 promoter; ICAM-2 promoter; Synthetic endothelial promoter; Endothelin-1 gene promoter; Amylase promoter; Insulin and human pdx-1 promoters; TRE-regulated insulin promoter; Enolase promoter; Enolase promoter; TRE-regulated synapsin promoter; Synapsin 1 promoter; PDGF-β promoter/CMV enhancer; PDGF-β, synapsin, tubulin-α and Ca2+/calmodulin-PK2 promoters combined with CMV enhancer; Phosphate-activated glutaminase and vesicular glutamate transporter-1 promoters; Glutamic acid decarboxylase-67 promoter; Tyrosine hydroxylase promoter; Neurofilament heavy gene promoter; Human red opsin promoter; Keratin-18 promoter; keratin-14 (K14) promoter; and Keratin-5 promoter.

49. The method of any one of claims 33-48, wherein one or more of the homologous recombination constructs further comprises a nucleotide sequence encoding a conditional destabilization domain or a conditional ribozyme switch.

50. A method of alleviating auxotrophy comprising:

(a) providing a plurality of auxotrophic progenitor cells which have been generated by knockout or knockdown of an auxotrophy-inducing gene comprising at least a first and a second independent functional domain; and

(b) inserting into the genome of the auxotrophic progenitor cells a first construct comprising an open reading frame of the first independent functional domain into a first tissue-specific gene locus, and inserting a second construct comprising an open reading frame of the second independent functional domain into a second tissue-specific gene locus, wherein expression of the tissue-specific genes at the first and second loci is not disrupted, thereby alleviating the auxotrophy upon differentiation of the progenitor cells into a cell type or tissue expressing the first and the second tissue-specific genes at the first and second loci.

51. A method of selecting cells having functionally integrated at least a first exogenous gene and a second exogenous gene, the method comprising:

(a) providing a plurality of cells with a knockout or knockdown of an auxotrophy-inducing gene comprising at least a first and a second independent functional domain, resulting in auxotrophy for an auxotrophic factor in the plurality of cells;

(b) growing the plurality of cells in a medium providing the auxotrophic factor;

(c) transfecting the plurality of cells with a first delivery system comprising a nucleotide sequence encoding the first exogenous gene and a nucleotide sequence encoding the first independent functional domain and a second delivery system comprising a nucleotide sequence encoding the second exogenous gene and a nucleotide sequence encoding the second independent functional domain; and

(d) replacing the medium with a medium lacking the auxotrophic factor, thereby selecting cells that have functionally integrated the first and second exogenous genes.

52. A method of generating a population of mature human beta cells comprising:

(a) contacting a first plurality of progenitor cells with a CRISPR/Cas system comprising a gRNA targeting biallelically a portion of a human UMPS gene resulting in a second plurality of progenitor cells, which is auxotrophic for uridine;

(b) contacting the second plurality of progenitor cells with a first homologous recombination construct and a second homologous recombination construct to generate a third plurality of progenitor cells, the first homologous recombination construct comprising a nucleotide sequence encoding insulin or an insulin-dependent expression control sequence operably linked to a first independent functional domain of UMPS, and the second homologous recombination construct comprising a nucleotide sequence encoding Nkx6.1 or an Nkx6.1-dependent expression control sequence operably linked to a second independent functional domain of UMPS, wherein the first and the second independent functional domains are selected from OPRT and ODC and are expressed only in progenitor cells expressing both insulin and Nkx6.1;

(c) contacting the third plurality of progenitor cells with uridine;

(d) stimulating differentiation of the plurality of progenitor cells into mature beta cells; and

(e) selecting for mature beta cells expressing both insulin and Nkx6.1 by removing uridine, thereby generating the population of mature human beta cells.

53. Use of the mature human beta cells produced by the method of claim 52 in the manufacture of a medicament for the treatment of type 1 diabetes.

54. A mature human beta cell selected from a population of in vitro differentiated progenitor cells, the mature human beta cell comprising a biallelic genetic modification of an auxotrophy-inducing gene resulting in auxotrophy for an auxotrophic factor and one or more transgenes re-expressing the auxotrophy-inducing gene or one or more independent functional domains of the auxotrophy-inducing gene.

55. A method of generating a sub-population of human cardiomyocytes comprising:

(a) contacting a first plurality of progenitor cells with a CRISPR/Cas system comprising a gRNA targeting biallelically a portion of a human UMPS gene resulting in a second plurality of the progenitor cells, which is auxotrophic for uridine;

(b) contacting the second plurality of progenitor cells with a first homologous recombination construct and a second homologous recombination construct to produce a third plurality of progenitor cells, the first homologous recombination construct comprising a nucleotide sequence encoding TBX5 or a TBX5-dependent expression control sequence operably linked to a first independent functional domain of UMPS, and the second homologous recombination construct comprising a nucleotide sequence encoding NKX2-5 or a NKX2-5-dependent expression control sequence operably linked to a second independent functional domain of UMPS, wherein the first and the second independent functional domains are selected from OPRT and ODC and are expressed only in progenitor cells expressing one or both of TBX5 and NKX2-5;

(c) contacting the third plurality of progenitor cells with uridine;

(d) stimulating differentiation of the plurality of progenitor cells into cardiomyocytes; and

(e) selecting for a sub-population of cardiomyocytes expressing one or both of TBX5 and NKX2-5 by removing uridine, thereby generating the sub-population of human cardiomyocytes.

56. The method of claim 55, wherein cells expressing:

(a) both TBX5 and NKX2-5 represent a sub-population comprising ventricular cardiomyocytes;

(b) TBX5 but not NKX2-5 represent a sub-population comprising nodal cardiomyocytes;

(c) not TBX5 but NKX2-5 represent a sub-population comprising atrial cardiomyocytes; and

(d) neither TBX5 nor NKX2-5 represent endothelial cells.

57. A cardiomyocyte selected from a population of in vitro differentiated cardiomyocytes comprising a biallelic genetic modification of an auxotrophy-inducing gene resulting in auxotrophy for an auxotrophic factor and one or more transgenes re-expressing the auxotrophy-inducing gene or one or more independent functional domains of the auxotrophy-inducing gene.

58. A method of generating a population of stable T reg cells comprising:

(a) contacting a first plurality of progenitor cells with a CRISPR/Cas system comprising a gRNA targeting biallelically a portion of a human UMPS gene resulting in a second plurality of progenitor cells, which isauxotrophic for uridine;

(b) contacting the second plurality of progenitor cells with a first homologous recombination construct and a second homologous recombination construct to produce a third plurality of progenitor cells, the first homologous recombination construct comprising a nucleotide sequence encoding FOXP3 or a FOXP3-dependent expression control sequence operably linked to a first independent functional domain of UMPS, and the second homologous recombination construct comprising a nucleotide sequence encoding a cell naïveté-associated promoter or an expression control sequence of a cell naïveté-associated promoter operably linked to a second independent functional domain of UMPS, wherein the first and the second independent functional domains are selected from OPRT and ODC and are expressed only in progenitor cells expressing both FOXP3 and a gene associated with the cell naïveté-associated promoter;

(c) contacting the third plurality of progenitor cells with uridine;

(d) stimulating differentiation of the plurality of progenitor cells into stable T reg cells;

and

(e) selecting for stable T reg cells expressing both FOXP3 and the gene associated with the cell naïveté-associated promoter by removing uridine, thereby generating the population of stable T reg cells.

59. The method of claim 58, wherein the cell naïveté-associated promoter is a promoter associated with PTPRC or CCR7.

60. Use of the stable T reg cells produced by the method of claim 58 or 59 in the manufacture of a medicament for the treatment of a disease, disorder, or condition in a subject, wherein the disease, disorder, or condition comprises an immune disease or cancer.

61. A population of stable T reg cells selected from a population T reg cells comprising a biallelic genetic modification of an auxotrophy-inducing gene resulting in auxotrophy for an auxotrophic factor and one or more transgenes re-expressing the auxotrophy-inducing gene or one or more independent functional domains of the auxotrophy-inducing gene.

62. A method of generating a population of cells having incorporated a first and a second expression cassette, the method comprising:

(a) culturing in the presence of uridine a plurality of cells genetically engineered to be auxotrophic for uridine;

(b) contacting the plurality of cells with a first expression construct and a second expression construct,

the first expression construct comprising a first expression cassette comprising a nucleotide sequence encoding a first payload and a second expression cassette comprising a nucleotide sequence encoding a first independent functional domain of UMPS, and

the second expression construct comprising a third expression cassette comprising a nucleotide sequence encoding a second payload and a fourth expression cassette

comprising a nucleotide sequence encoding a second independent functional domain of UMPS; and

(c) withdrawing the uridine from the plurality of cells, thereby generating the population of cells having incorporated a first and a second expression cassette.

63. The method of claim 62, wherein the first expression construct is a homologous recombination construct targeting a specific genetic locus.

64. The method of claim 62 or 63, wherein the second expression construct is a homologous recombination construct targeting a specific genetic locus.

65. The method of claim 63 or 64, wherein the specific genetic locus is a safe harbor locus.

66. The method of claim 65, wherein the safe harbor locus is CCR5.

67. The method of any one of claims 62-66, wherein the plurality of cells genetically engineered to be auxotrophic for uridine comprises UMPS knockout cells.

68. The method of any one of claims 62-67, wherein the plurality of cells is derived from progenitor cells.

69. The method of any one of claims 62-68, wherein the nucleotide sequence encoding the first payload is under the transcriptional control of a tissue-specific promoter.

70. The method of any one of claims 62-68, wherein the nucleotide sequence encoding the second payload is under the transcriptional control of a tissue-specific promoter.

71. The method of any one of claims 62-70, wherein the nucleotide sequence encoding the first payload and the nucleotide sequence encoding the second payload are each under the transcriptional control of a tissue-specific promoter.

72. The method of any one of claims 62-71, wherein the nucleotide sequence encoding the first independent functional domain of UMPS is under the transcriptional control of a constitutive promoter.

73. The method of any one of claims 62-71, wherein the nucleotide sequence encoding the second independent functional domain of UMPS is under the transcriptional control of a constitutive promoter.

74. The method of any one of claims 62-73, wherein the nucleotide sequences encoding the first and the second independent functional domains of UMPS are each under the transcriptional control of a constitutive promoter.

75. The method of any one of claims 62-74, wherein the first and the second independent functional domains of UMPS are independently selected from OPRT and ODC.

76. The method of any one of claims 69-71, further comprising differentiating the cells in vitro to a desired cell type.

77. The method of claim 76, wherein the tissue-specific promoter is a megakaryocyte-specific promoter and the desired cell type is a megakaryocyte.

78. The method of claim 76 or 77, wherein differentiating the cells to the desired cell type leads to expression of the first payload, the second payload, or the first and the second payload.

79. A population of cells comprising a first and a second expression cassette generated by the method of any one of claims 62-77.

80. An engineered cell comprising a knockout of an auxotrophy-inducing gene and a first expression construct and a second expression construct, wherein the first expression construct and the second expression construct are stably integrated into the genome of the cell, and wherein the first expression construct and the second expression construct each comprises a nucleotide sequence encoding a first and a second independent functional domain of the auxotrophy-inducing gene.

81. A method of generating megakaryocytes in vitro comprising:

(a) culturing in the presence of an auxotrophic factor a plurality of progenitor cells genetically engineered to be auxotrophic for the auxotrophic factor;

(b) differentiating the cells to megakaryocytes; and

(c) withdrawing the auxotrophic factor.

82. The method of claim 81, wherein the plurality of cells comprises progenitor cells.

83. The method of claim 81 or 82, wherein the plurality of cells comprises UMPS knockout cells.

84. The method of any one of claims 81-83, wherein the auxotrophic factor is uridine.

85. The method of any one of claims 81-83, wherein withdrawing the uridine causes proliferative cells to die or to fail to propagate.

86. The method of any one of claims 81-84, wherein the megakaryocytes generate platelets.

87. The method of any one of claims 81-86, wherein the platelets persist after withdrawing the auxotrophic factor.

88. The method of any one of claims 81-87, wherein a substantially pure population of platelets is generated.

89. A substantially pure population of platelets generated by the method of any one of claims 81-88.

90. A substantially pure population of platelets generated in vitro from a plurality of cells genetically engineered to be auxotrophic.

91. A method of generating a population of engineered platelets comprising:

(a) culturing in the presence of an auxotrophic factor a plurality of cells genetically engineered to be auxotrophic for the auxotrophic factor, the plurality of cells having a knockout of an auxotrophy-inducing gene;

(b) contacting the plurality of cells with a first expression construct and a second expression construct,

the first expression construct comprising a first expression cassette comprising a nucleotide sequence encoding a first payload and a second expression cassette comprising a nucleotide sequence encoding a first independent functional domain of the auxotrophy-inducing gene, and

the second expression construct comprising a third expression cassette comprising a nucleotide sequence encoding a second payload and a fourth expression cassette comprising a nucleotide sequence encoding a second independent functional domain of the auxotrophy-inducing gene; and

(c) withdrawing the uridine from the plurality of cells.

92. The method of claim 91, wherein the first expression, the second expression construct, or the first and the second expression construct is a homologous recombination construct targeting a specific genetic locus.

93. The method of claim 92, wherein the specific genetic locus is a safe harbor locus.

94. The method of claim 93, wherein the safe harbor locus is CCR5.

95. The method of any one of claims 91-94, wherein the auxotrophy-inducing gene is UMPS and the auxotrophic factor is uridine.

96. The method of claim 95, wherein the first and the second independent functional domains are selected from OPRT and ODC.

97. The method of any one of claims 91-96, wherein the plurality of cells is derived from progenitor cells.

98. The method of any one of claims 91-97, wherein the nucleotide sequence encoding the first payload is under the transcriptional control of a tissue-specific promoter.

99. The method of any one of claims 91-97, wherein the nucleotide sequence encoding the second payload is under the transcriptional control of a tissue-specific promoter.

100. The method of any one of claims 91-99, wherein the nucleotide sequence encoding the first payload and the nucleotide sequence encoding the second payload are each under the transcriptional control of a tissue-specific promoter.

101. The method of any one of claims 91-100, wherein the nucleotide sequence encoding the first independent functional domain is under the transcriptional control of a constitutive promoter.

102. The method of any one of claims 91-100, wherein the nucleotide sequence encoding the second independent functional domain is under the transcriptional control of a constitutive promoter.

103. The method of any one of claims 91-102, wherein the nucleotide sequences encoding the first and the second independent functional domains are each under the transcriptional control of a constitutive promoter.

104. The method of any one of claims 91-103, further comprising differentiating the cells in vitro to a desired cell type.

105. The method of claim 104, wherein the tissue-specific promoter is a megakaryocyte-specific promoter and the desired cell type is a megakaryocyte.

106. The method of any one of claims 104 or 105, wherein differentiating the cells to the desired cell type leads to expression of the first payload, the second payload, or the first and the second payload.

107. The method of claim 105 or 106, wherein the megakaryocytes produce platelets.

108. The method of claim 107, wherein the platelets are loaded with the first payload, the second payload, or the first and the second payload.

109. The method of any one of claims 104-108, wherein the differentiating the cells in vitro is in the presence of the auxotrophic factor.

110. The method of any one of claims 107, wherein the differentiated platelets do not express the first and the second independent functional domains.

111. The method of claim 110, further comprising adding 5-FOA.

112. The method of any one of claims 104-111, further comprising withdrawing the auxotrophic factor after differentiating the cells, wherein remaining nucleated, proliferating cells die or fail to propagate upon withdrawal of the auxotrophic factor.

113. An engineered cell comprising a knockout of UMPS, a first expression construct and a second expression construct, wherein the first expression construct and the second expression construct are stably integrated into the genome of the cell, and wherein the first expression construct and the second expression construct each comprises a nucleotide sequence encoding a first and a second independent functional domain of UMPS selected from OPRT and ODC.

114. A substantially pure population of platelets prepared in vitro from cells engineered to be UMPS knockout cells.

115. A kit comprising materials for performing a method of any one of claims 1-52, 55-56, 58-59, 62,-78, 81-88 or 91-112.

116. Use of the differentiated cells produced by a method of any one of claims 1-52, 55-56, 58-59, 62,-78, 81-88 or 91-112 in the manufacture of a medicament for the treatment of a disease, wherein the differentiated cells are immune cells.