blood cells from a defined, well-characterized, source. Additionally, RBCs produced by the novel methods of the present disclosure may also contain and/or express one or more recombinant protein of interest that can be used to treat a subject in need thereof.

[0013] Accordingly, certain examples of the present disclosure relate to a method for producing a population of mature red blood cells from hematopoietic stem cells, by: culturing the hematopoietic stem cells in the presence of EPO and one or more first recombinant protein, or biologically active fragment thereof, that promotes cell survival and/or proliferation; under conditions that induce differentiation of the hematopoietic stem cells to mature red bloods cells, thereby producing a population of mature red blood cells. In one aspect, the present disclosure refers to a method for producing a population of mature red blood cells from hematopoietic stem cells, comprising: culturing hematopoietic stem cells in a culture media comprising EPO and one or more first recombinant protein that promotes cell survival and proliferation under conditions that induce differentiation of the hematopoietic stem cells to mature red bloods cells, thereby producing a population of mature red blood cells, wherein the first recombinant protein comprises a MYC polypeptide or a biologically active fragment thereof. In some examples, the hematopoietic stem cells are conditionally immortalized hematopoietic stem cells. In some examples that may be combined with any of the preceding examples, the hematopoietic stem cells are protein transduced hematopoietic stem cells, and the one or more first recombinant protein, or biologically active fragment thereof, is an exogenous protein. In some examples that may be combined with any of the preceding examples, the hematopoietic stem cells are transgenic hematopoietic stem cells. In some examples that may be combined with any of the preceding examples, the one or more first recombinant protein, or biologically active fragment thereof, is one or more polypeptide selected from a MYC polypeptide, an ICN-1 polypeptide, homologues thereof, and biologically active fragments thereof. In some examples that may be combined with any of the preceding examples, the MYC polypeptide is one or more MYC polypeptide selected from n-Myc, c-Myc, l-Myc, v-Myc, and s-Myc. In some examples that may be combined with any of the preceding examples, one or more of the one or more first recombinant protein, or biologically active fragment thereof, contains a protein transduction domain. In some examples that may be combined with any of the preceding examples, the protein transduction domain is one or more protein transduction domain selected from TAT, VPR, and EPTD. In some examples that may be combined with any of the preceding examples, the one or more first recombinant protein, or biologically active fragment thereof, is TAT-MYC. In some examples that may be combined with any of the preceding examples, the one or more first recombinant protein, or biologically active fragment thereof, is provided as a bolus. In some examples that may be combined with any of the preceding examples, the one or more first recombinant protein is provided as a bolus about every 24 hours, about every 48 hours, or about every 72 hours. In some examples that may be combined with any of the preceding examples, the method

further includes culturing the hematopoietic stem cells in the presence of IL-3. In some examples that may be combined with any of the preceding examples, the method further includes culturing the hematopoietic stem cells in the absence of feeder cells and serum. In some examples that may be combined with any of the preceding examples, the method further includes culturing the population of mature red blood cells in the presence of one or more second recombinant protein, or biologically active fragment thereof, that inhibits apoptosis. In some examples that may be combined with any of the preceding examples, the one or more second recombinant protein, or biologically active fragment thereof, contains one or more Bcl-2 homology domains. In some examples that may be combined with any of the preceding examples, the one or more Bcl-2 homology domains are one or more Bcl-2 homology domains selected from BH1, BH2, BH3, and BH4. In some examples that may be combined with any of the preceding examples, the one or more second recombinant protein is one or more of Bcl-2, Bcl-w, Bcl-X, Bcl-XL, or Mcl-1. In some examples that may be combined with any of the preceding examples, the one or more second recombinant protein, or biologically active fragment thereof, is Bcl-2. In some examples that may be combined with any of the preceding examples, one or more of the one or more second recombinant protein, or biologically active fragment thereof, contains a protein transduction domain. In some examples that may be combined with any of the preceding examples, the protein transduction domain is one or more protein transduction domains selected from TAT, VPR, and EPTD. In some examples that may be combined with any of the preceding examples, the one or more second recombinant protein, or biologically active fragment thereof, is TAT-Bcl-2. In some examples that may be combined with any of the preceding examples, the hematopoietic stem cells further contain one or more recombinant protein of interest, or biologically active fragment thereof. In some examples that may be combined with any of the preceding examples, the one or more recombinant protein of interest, or biologically active fragment thereof, is an exogenous protein, or biologically active fragment thereof. In some examples that may be combined with any of the preceding examples, the hematopoietic stem cells contain one or more transgenes that encode one or more proteins selected from the one or more first recombinant protein, or biologically active fragment thereof; the one or more second recombinant protein, or biologically active fragment thereof; and one or more recombinant protein of interest, or biologically active fragment thereof. In some examples that may be combined with any of the preceding examples, the expression or function of one or more of the one or more first recombinant protein, or biologically active fragment thereof; the one

or more second recombinant protein, or biologically active fragment thereof; or one or more recombinant protein of interest, or biologically active fragment thereof is controllable. In some examples that may be combined with any of the preceding examples, the expression or function of one or more of the one or more first recombinant protein, or biologically active fragment thereof; the one or more second recombinant protein, or biologically active fragment thereof; or one or more recombinant protein of interest, or biologically active fragment thereof is inducible. In some examples that may be combined with any of the preceding examples, one or more of the one or more transgenes encode an antibiotic responsive element or a hormone responsive element. In some examples that may be combined with any of the preceding examples, the antibiotic responsive element or the hormone responsive element is one or more responsive element selected from an estrogen response element, a gonadotropin response element, or a tetracycline response element, and a glucocorticoid response element. In some examples that may be combined with any of the preceding examples, the one or more transgenes over-express one or more one or more proteins selected from the one or more first recombinant protein, or biologically active fragment thereof; the one or more second recombinant protein, or biologically active fragment thereof; and one or more recombinant protein of interest, or biologically active fragment thereof. In some examples that may be combined with any of the preceding examples, the production of the population of mature red blood cells is accelerated by at least 45% compared to production of a population of red blood cells from a primary stem cell cultured in the presence of IL-3 and EPO for eight days, then in the presence of feeder cells and EPO for three days, and finally in the presence of feeder cells alone for 10 days. In some examples that may be combined with any of the preceding examples, the population of mature red blood cells is produced in about 7 to 14 days. In some examples that may be combined with any of the preceding examples, the population of mature red blood cells exhibits one or more characteristics selected from a population of mature red blood cells, where about 40% to about 100% of the cells are anucleated; a population of mature red blood cells, where about 40% to about 100% of the cells express GPA; a population of mature red blood cells, where about 40% to about 100% of the cells express adult hemoglobin; a population of mature red blood cells, where about 40% to about 100% of the cells exhibit decreased levels of CD71 expression; a population of mature red blood cells, where about 40% to about 100% of the cells exhibit decreased levels of fetal hemoglobin expression. In some examples that may be combined with any of the preceding examples, the hematopoietic stem cells are human hematopoietic stem cells. In

some examples that may be combined with any of the preceding examples, the hematopoietic stem cells were isolated from a patient with a rare blood type. In some examples that may be combined with any of the preceding examples, the patient has an autoimmune condition. In some examples that may be combined with any of the preceding examples, the hematopoietic stem cells were produced from embryonic stem cells or induced pluripotent stem cells. In some examples that may be combined with any of the preceding examples, the population of mature red blood cells is a population of human cells. In some examples that may be combined with any of the preceding examples, the population of mature red blood cells has one or more blood types selected from A^+, A^-, B^+, B^-, AB^+, AB^-, O^+, and O^-. In some examples that may be combined with any of the preceding examples, the population of mature red blood cells is a rare blood type. In some examples that may be combined with any of the preceding examples, the population of mature red blood cells is a population of non-human animal cells.

[0014] Other examples of the present disclosure relate to a population of *in vitro* differentiated mature red blood cells, containing anucleated red blood cells expressing GPA, expressing adult hemoglobin, exhibiting decreased levels of CD71 expression, and exhibiting decreased levels of fetal hemoglobin expression, where: about 40% to about 100% of the red blood cells in the population are anucleated; about 40% to about 100% of the red blood cells in the population express GPA; about 40% to about 100% of the red blood cells in the population express adult hemoglobin; about 40% to about 100% of the red blood cells in the population exhibit decreased levels of CD71 expression; and about 40% to about 100% of the red blood cells in the population exhibit decreased levels of fetal hemoglobin expression. In some examples that may be combined with any of the preceding examples, the red blood cells contain one or more recombinant protein of interest. In some examples that may be combined with any of the preceding examples, the red blood cells have a rare blood type. In some examples that may be combined with any of the preceding examples, the red blood cells are human red blood cells. In some examples that may be combined with any of the preceding examples, the red blood cells are non-human animal red blood cells. In some examples that may be combined with any of the preceding examples, the population of *in vitro* differentiated mature red blood cells is produced by any methods of producing a population of mature red blood cells from hematopoietic stem cells of any of the preceding examples.

[0015] Other examples of the present disclosure relate to a pharmaceutical composition containing: a population of *in vitro* differentiated mature red blood cells, where about 40% to about 100% of the red blood cells in the population are anucleated, about 40% to about 100% of the red blood cells in the population express GPA, about 40% to about 100% of the red blood cells in the population express adult hemoglobin, about 40% to about 100% of the red blood cells in the population exhibit decreased levels of CD71 expression, and about 40% to about 100% of the red blood cells in the population exhibit decreased levels of fetal hemoglobin expression; and one or more pharmaceutically acceptable excipients. In some examples that may be combined with any of the preceding examples, where the composition further contains one or more recombinant protein of interest, or biologically active fragment thereof. In some examples that may be combined with any of the preceding examples, the population of red blood cells contains one or more recombinant protein of interest, or biologically active fragment thereof. In some examples that may be combined with any of the preceding examples, the one or more recombinant protein of interest, or biologically active fragment thereof, is an exogenous protein. In some examples that may be combined with any of the preceding examples, the red blood cells have a rare blood type. In some examples that may be combined with any of the preceding examples, the red blood cells are human red blood cells. In some examples that may be combined with any of the preceding examples, the red blood cells are non-human animal red blood cells. In some examples that may be combined with any of the preceding examples, the population of *in vitro* differentiated mature red blood cells is produced by any methods of producing a population of mature red blood cells from hematopoietic stem cells of any of the preceding examples.

[0016] Other examples of the present disclosure relate to a method of treatment, prevention, or diagnosis of a disease or disorder characterized by a deficiency of anucleated red blood cells by: providing to a subject in need thereof a population of *in vitro* differentiated mature red blood cells, where about 40% to about 100% of the red blood cells in the population are anucleated, about 40% to about 100% of the red blood cells in the population express GPA, about 40% to about 100% of the red blood cells in the population express adult hemoglobin, about 40% to about 100% of the red blood cells in the population exhibit decreased levels of CD71 expression, and about 40% to about 100% of the red blood cells in the population exhibit decreased levels of fetal hemoglobin expression. In some examples that may be combined with any of the preceding examples, the subject is a human. In some

examples that may be combined with any of the preceding examples, the subject is a non-human animal. In some examples that may be combined with any of the preceding examples, the population of red blood cells contains one or more recombinant protein of interest, or a biologically active fragment thereof. In some examples that may be combined with any of the preceding examples, the one or more recombinant protein of interest, or biologically active fragment thereof, is an exogenous protein. In some examples that may be combined with any of the preceding examples, the population of *in vitro* differentiated mature red blood cells are produced by any methods of producing a population of mature red blood cells from hematopoietic stem cells of any of the preceding examples.

[0017] Other examples of the present disclosure relate to a method for extending the half-life of a population of mature red blood cells *in vitro* by: maintaining the population of red blood cells in a media containing one or more exogenous polypeptide, homologues thereof or biologically active fragments thereof, that inhibit apoptosis. In some examples that may be combined with any of the preceding examples, the one or more exogenous polypeptide, homologues thereof or biologically active fragments thereof, contains one or more Bcl2 homology domains. In some examples that may be combined with any of the preceding examples, the one or more Bcl-2 homology domains are one or more Bcl-2 homology domains selected from BH1, BH2, BH3, and BH4. In some examples that may be combined with any of the preceding examples, the one or more exogenous polypeptide, homologues thereof or biologically active fragments thereof, is one or more of Bcl-2, Bcl-w, Bcl-X, Bcl-XL, or Mcl-1. In some examples that may be combined with any of the preceding examples, the one or more exogenous polypeptide, homologues thereof or biologically active fragments thereof, is Bcl-2. In some examples that may be combined with any of the preceding examples, one or more of the one or more exogenous polypeptide, homologues thereof or biologically active fragments thereof, contains a protein transduction domain. In some examples that may be combined with any of the preceding examples, the protein transduction domain is one or more protein transduction domains selected from TAT, VPR, and EPTD. In some examples that may be combined with any of the preceding examples, the one or more exogenous polypeptide, homologues thereof or biologically active fragments thereof, is TAT-Bcl-2. In some examples that may be combined with any of the preceding examples, the population of mature red blood cells is

produced by any methods of producing a population of mature red blood cells from hematopoietic stem cells of any of the preceding examples.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] **Figure 1** depicts a general approach for the generation of ctlt-HSC cell lines. **Figure 1A** depicts a schematic representation of retroviral constructs used to transduce primary murine HSC cells with MYC-ER and Bcl-2. **Figure 1B** depicts flow cytometric analyses of HSCs obtained from 5-fluorouracil treated donors following transduction with pMIG-MYC-ER and pMIG-Bcl-2. **Figure 1C** depicts kinetics of leukemogenesis in mice transplanted with the transduced HSCs shown in Figure 1B. **Figure 1D** depicts FACS analysis of a ctlt-HSC soon after recovery from the bone marrow of leukemic mice. **Figure 1E** depicts FACS analysis of a ctlt-HSC cell line that has been established. **Figure 1F** depicts FACS analysis of stem cell markers in normal, unmanipulated Lt-HSCs from the bone marrow of wild type C57/BL6 mice.

[0019] **Figure 2** depicts the characterization of mature immune cells that arise following transplantation of ctlt-HSCs. **Figure 2A** depicts the frequency of cells derived from ctlt-HSCs in lymphoid tissues after transplantation. **Figure 2B** depicts the detection of GFP^+ myeloid lineage cells in the bone marrow. **Figure 2C** depicts the analysis of peripheral, mature lymphocytes in the spleen. **Figure 2D** depicts the analysis of peripheral, mature lymphocytes in transplant recipient mice following the second serial passage of ctlt-HSCs.

[0020] **Figure 3** depicts the surface phenotype of three human ctlt-HSC cell lines. **Figure 3A**, **Figure 3B**, and **Figure 3C** show the $CD34^+$ fraction of transduced (i.e., GFP^+) HSCs from three established human ctlt-HSC cell lines. **Figure 3D**, **Figure 3E**, and **Figure 3F** show the c-kit^+ fraction of transduced (i.e., GFP^+) HSCs from three established human ctlt-HSC cell lines. **Figure 3G** depicts that the transduced (i.e., GFP^+) HSCs from the established human ctlt-HSC cell lines do not express CD45. **Figure 3H** depicts that the transduced (i.e., GFP^+) HSCs from the established human ctlt-HSC cell lines do not express Flk-2. **Figure 3I** depicts that the transduced (i.e., GFP^+) HSCs from the established human ctlt-HSC cell lines do not express CD150.

[0021] **Figure 4** depicts the differentiation of human ctlt-HSC cells lines into mature lymphoid cells in NOD/SCID/ß2$M^{-/-}$ mice. **Figure 4A** depicts data obtained from a control mouse (no transplant). **Figure 4B**, **Figure 4C**, and **Figure 4D** show data obtained from a

What is claimed is:

1. A method for producing a population of mature red blood cells from hematopoietic stem cells, comprising:

culturing hematopoietic stem cells in a culture media comprising EPO and one or more first recombinant protein that promotes cell survival and proliferation under conditions that induce differentiation of the hematopoietic stem cells to mature red bloods cells, thereby producing a population of mature red blood cells,

wherein the first recombinant protein comprises a MYC polypeptide or a biologically active fragment thereof.

2. The method of claim 1, wherein the MYC polypeptide or biologically active fragment thereof, is TAT-MYC.

3. The method of any one of claims 1 or 2, further comprising culturing the population of mature red blood cells in the presence of one or more second recombinant protein, or biologically active fragment thereof, that inhibits apoptosis.

4. The method of claim 3, wherein the one or more second recombinant protein, or biologically active fragment thereof, comprises one or more Bcl2 homology domains selected from the group consisting of BH1, BH2, BH3, and BH4.

5. The method of any one of claims 1 to 4, wherein the hematopoietic stem cells further comprise one or more recombinant proteins of interest, or biologically active fragment thereof.

6. The method of any one of claims 1 to 5, wherein the hematopoietic stem cells comprise one or more transgenes that encode one or more recombinant proteins of interest, or biologically active fragment thereof.

7. The method of either one of claims 5 or 6, wherein the expression or function of one or more recombinant proteins of interest, or biologically active fragment thereof is controllable or inducible.

8. The method of any one of claims 1 to 7, wherein the population of mature red blood cells is produced in about 7 to 14 days.

9. The method of any one of claims 1 to 8, wherein the population of mature red blood cells exhibits one or more characteristics selected from the group consisting of a population of mature red blood cells, wherein about 40% to about 100% of the cells are anucleated; a population of mature red blood cells, wherein about 40% to about 100% of the cells express GPA; a population of mature red blood cells, wherein about 40% to about 100% of the cells express adult hemoglobin; a population of mature red blood cells, wherein about 40% to about 100% of the cells exhibit decreased levels of CD71 expression; a population of mature red blood cells, wherein about 40% to about 100% of the cells exhibit decreased levels of fetal hemoglobin expression.