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(54) Title: COMPOSITIONS AND METHODS RELATED TO THERAPEUTIC CELL SYSTEMS EXPRESSING EXOGENOUS RNA

(57) Abstract: The invention includes compositions and methods related to erythroid cells comprising exogenous RNA encoding a protein. The exogenous RNA can comprise a heterologous untranslated region comprising a regulatory element. Alternatively or in combination, the exogenous RNA can comprise chemical modifications.

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COMPOSITIONS AND METHODS RELATED TO THERAPEUTIC CELL SYSTEMS EXPRESSING EXOGENOUS RNA

RELATED APPLICATIONS

This application claims priority to U.S. Serial No. 62/359416 filed July 7, 2016, the contents of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 7, 2017, is named R2081-7015WO_SL.txt and is 921 bytes in size.

BACKGROUND

Red blood cells have been considered for use as drug delivery systems, e.g., to degrade toxic metabolites or inactivate xenobiotics, and in other biomedical applications. There is a need in the art for improved red blood cell based drug delivery systems.

SUMMARY OF THE INVENTION

The invention includes compositions and methods related to erythroid cells comprising exogenous RNA (e.g., exogenous RNA encoding a protein). The exogenous RNA can comprise a coding region and a heterologous untranslated region (UTR), e.g., a UTR comprising a regulatory element. Alternatively or in combination, the exogenous RNA can comprise chemical modifications. Alternatively or in combination, the exogenous RNA can be a regulatory RNA such as a miRNA. While not wishing to be bound by theory, in some embodiments the exogenous RNA has improved parameters, such as stability or increased translation, relative to a control.

In certain aspects, the present disclosure provides an enucleated erythroid cell comprising: an exogenous mRNA comprising a coding region operatively linked to a heterologous untranslated region (UTR).

In certain aspects, the present disclosure provides an enucleated erythroid cell, comprising: an exogenous mRNA comprising a coding region operatively linked to a heterologous untranslated region (UTR), wherein the heterologous UTR comprises a regulatory element.

In certain aspects, the present disclosure provides an erythroid cell, e.g., an enucleated erythroid cell, comprising an exogenous mRNA that comprises one or more chemically modified nucleotides (e.g., one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof).

The disclosure also provides a method of producing an erythroid cell, e.g., enucleated erythroid cell, comprising:

a) contacting an erythroid cell, e.g., a nucleated erythroid cell, with an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element, (e.g., isolated RNA or in vitro transcribed RNA), and

b) maintaining the contacted erythroid cell under conditions suitable for uptake of the exogenous mRNA,

thereby producing the erythroid cell, e.g., an enucleated erythroid cell.

The disclosure also provides a method of producing an erythroid cell, e.g., enucleated erythroid cell, comprising:

a) contacting an erythroid cell, e.g., a nucleated erythroid cell, with an exogenous mRNA comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof; and

b) maintaining the contacted erythroid cell under conditions suitable for uptake of the exogenous mRNA,

thereby producing the erythroid cell, e.g., an enucleated erythroid cell.

The disclosure further provides a method of producing an exogenous protein in an enucleated erythroid cell:

a) providing an erythroid cell, e.g., a nucleated erythroid cell, comprising an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element, (e.g., isolated RNA or in vitro transcribed RNA), and

b) culturing the erythroid cell under conditions suitable for production of the exogenous protein,

thereby producing the exogenous protein.

The disclosure further provides a method of producing an exogenous protein in an enucleated erythroid cell:

a) providing an erythroid cell, e.g., a nucleated erythroid cell, comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof, and

b) culturing the erythroid cell under conditions suitable for production of the exogenous protein,

thereby producing the exogenous protein.

In certain aspects, the disclosure provides a method of providing a subject with an exogenous protein, providing a subject with an enucleated erythroid cell which can produce an exogenous protein, or treating a subject, comprising administering to the subject:

an erythroid cell, e.g., a nucleated erythroid cell, comprising an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element, (e.g., isolated RNA or in vitro transcribed RNA),

thereby providing a subject with an exogenous protein, providing a subject with an enucleated erythroid cell which can produce an exogenous protein, or treating a subject.

In certain aspects, the disclosure provides a method of providing a subject with an exogenous protein, providing a subject with an enucleated erythroid cell which can produce an exogenous protein, or treating a subject, comprising administering to the subject:

an erythroid cell, e.g., a nucleated erythroid cell, comprising an exogenous mRNA comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof,

thereby providing a subject with an exogenous protein, providing a subject with an enucleated erythroid cell which can produce an exogenous protein, or treating a subject.

In some aspects, the disclosure provides a method of evaluating an erythroid cell, e.g., enucleated erythroid cell (or a batch of such cells) comprising:

a) providing an erythroid cell, e.g., a nucleated erythroid cell, comprising an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element (or a batch of such cells), and

b) evaluating the erythroid cell, e.g., the nucleated erythroid cell (or batch of such cells) for a preselected parameter,

thereby evaluating the erythroid cell, e.g., enucleated erythroid cell (or a batch of such cells).

In some aspects, the disclosure provides a method of evaluating an erythroid cell, e.g., enucleated erythroid cell (or a batch of such cells) comprising:

a) providing an erythroid cell, e.g., a nucleated erythroid cell, comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof, and

b) evaluating the erythroid cell, e.g., the nucleated erythroid cell (or batch of such cells) for a preselected parameter,

thereby evaluating the erythroid cell, e.g., enucleated erythroid cell (or a batch of such cells).

In certain aspects, the present disclosure provides a method of producing a plurality of enucleated erythroid cells comprising an exogenous protein, comprising: a) contacting an erythroid cell with an exogenous mRNA comprising a coding region and a heterologous UTR, (e.g., isolated RNA or in vitro transcribed RNA), and b) culturing the erythroid cell under conditions suitable for production of the exogenous protein, thereby producing the enucleated erythroid cell comprising the exogenous protein.

In some aspects, the present disclosure provides an enucleated erythroid cell comprising an exogenous mRNA comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, or one or more chemically modified caps of Table 3, or any combination thereof.

In some aspects, the present disclosure provides a method of producing a plurality of enucleated erythroid cells comprising an exogenous protein, comprising:

a) contacting an erythroid cell with an exogenous mRNA described herein (e.g., isolated RNA or in vitro transcribed RNA) that encodes an exogenous protein, wherein the exogenous mRNA comprises one or more chemically modified nucleotides of Table 1, one or more

chemical backbone modifications of Table 2, or one or more chemically modified caps of Table 3, or any combination thereof, and

b) culturing the erythroid cell under conditions suitable for production of the exogenous protein,

thereby producing the enucleated erythroid cell comprising the exogenous protein.

In certain aspects, the present disclosure provides an RNA molecule comprising: a) a coding region that encodes a red blood cell transmembrane protein, e.g., GPA or Kell, and b) a heterologous UTR, e.g., a UTR comprising one or more regulatory elements or a hemoglobin 3' UTR.

In certain aspects, the present disclosure provides an RNA molecule comprising: a) a coding region that encodes a red blood cell transmembrane protein, e.g., GPA or Kell, and b) one or more modified nucleotides described herein, e.g., a nucleotide of Table 1, 2, or 3.

In some aspects, the present disclosure provides a method of producing an erythroid cell described herein, providing contacting an erythroid cell, e.g., an erythroid cell precursor, with one or more nucleic acids described herein and placing the cell in conditions that allow expression of the nucleic acid.

In some aspects, the present disclosure provides a preparation, e.g., pharmaceutical preparation, comprising a plurality of erythroid cells described herein, e.g., at least 10^8, 10^9, 10^{10}, 10^{11}, or 10^{12} cells.

In some aspects, the disclosure provides a method of contacting erythroid cells with an exogenous mRNA during maturation phase, e.g., during day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 of maturation phase.

Any of the aspects herein, e.g., the aspects above, can be characterized by one or more of the embodiments herein, e.g., the embodiments below.

In some embodiments, the methods herein comprise a step of:

c) culturing the erythroid cell subsequent to uptake of the exogenous RNA,

In embodiments, the UTR occurs naturally operatively linked to a coding region other than the subject coding region, or has at least at least 70, 80, 90, 95, 99, or 100% homology to such naturally occurring UTR. In embodiments, the UTR does not occur naturally with the subject coding region, e.g., differs by at least 1 nucleotide, e.g., by at least 1, 2, 3, 4, 5, 10, 20,

25, 30, 35, 40, 45, or 50 % of its nucleotides, from the UTR which occurs naturally operatively linked with the subject coding region. In embodiments, the UTR does not exist in nature.

In embodiments, the UTR comprises a 3' UTR. In embodiments, the UTR comprises a 5' UTR. In embodiments, the heterologous UTR is a 5' UTR, and the exogenous mRNA further comprises a heterologous 3' UTR. In embodiments, the UTR comprises a region that corresponds to an intron. In some embodiments, the RNA is capable of undergoing alternative splicing, e.g., encodes a plurality of splice isoforms. In embodiments, the alternative splicing comprises exon skipping, alternative 5' donor site usage, alternative 3' acceptor site usage, or intron retention. In an embodiment, the UTR comprises an intron in the coding region. In an embodiment, an intron in the coding region comprises the UTR. In an embodiment, the UTR is a 5' UTR that comprises an intron.

In embodiments, the enucleated erythroid cell further comprises a second UTR. In embodiments, the enucleated erythroid comprises a 3' UTR and a 5' UTR.

In embodiments, the UTR occurs naturally in a wild-type human cell. In embodiments, the UTR does not occur naturally in a wild-type human cell.

In embodiments, the coding region occurs naturally in a wild-type human cell and/or encodes a protein that occurs naturally in a wild-type human cell. In embodiments, the coding region does not occur naturally in a wild-type human cell and/or encodes a protein that does not occur naturally in a wild-type human cell. In embodiments, the UTR occurs naturally operatively linked with a coding region that is expressed in a wild-type erythroid cell, e.g., a hemoglobin coding region.

In embodiments, the UTR is a globin UTR, e.g., a hemoglobin UTR, e.g., having the sequence of SEQ ID NO: 1 or a sequence with at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

In embodiments, the coding region encodes an enzyme, antibody molecule, complement regulatory protein, chelator, or a protein listed in Table 4. In some embodiments, the exogenous polypeptide comprises phenylalanine ammonia lyase (PAL) or a phenylalanine-metabolizing fragment or variant thereof.

In embodiments, the cell further comprises a protein encoded by the exogenous mRNA. In embodiments, the cell does not comprise DNA encoding the exogenous mRNA.

In some embodiments, the cell has not been or is not hypotonically loaded.

In embodiments, the exogenous mRNA comprises one or more chemically modified nucleotides, chemical backbone modifications, or modified caps, or any combination thereof. In embodiments, at least 50%, 60%, 70%, 80%, or 85% of the cells in the plurality produce the exogenous protein. In embodiments, the cell population has at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% cell viability.

In embodiments, the method comprises performing transfection, electroporation, hypotonic loading, change in cell pressure, cell deformation (e.g., CellSqueeze), or other method for disrupting the cell membrane to allow the exogenous RNA to enter the cell.

In embodiments, the exogenous mRNA has a half-life that is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% greater, or 2-fold, 5-fold, 10-fold, 20-fold, or 100-fold greater than the half-life of a corresponding mRNA lacking the chemical modification in a similar erythroid cell. In embodiments, the exogenous mRNA is present in the erythroid cell at a level that is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% greater, or 2-fold, 5-fold, 10-fold, 20-fold, or 100-fold greater than the level of an mRNA of identical sequence that lacks the chemical modification, in an otherwise similar erythroid cell, when measured at a similar timepoint after introduction of the mRNA.

In embodiments, the exogenous protein is present in the erythroid cell at a level that is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% greater, or 2-fold, 5-fold, 10-fold, 20-fold, or 100-fold greater than the level of protein produced by an mRNA of identical sequence that lacks the chemical modification, in an otherwise similar erythroid cell, when measured at a similar timepoint after introduction of the mRNA. In embodiments, the timepoint is 1, 2, 3, 4, 5, 6, 7, 14, 21, or 28 days after the cell is contacted with the mRNA. In embodiments, the chemical modification comprises a pseudouridine. In embodiments, the mRNA further comprises a cap. In embodiments, the mRNA further comprises a polyA tail.;

In embodiments, the exogenous protein is present in the erythroid cell at a level that is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% greater, or 2-fold, 5-fold, 10-fold, 20-fold, or 100-fold greater than the level of protein produced by an mRNA of identical sequence that lacks a polyA tail, in an otherwise similar erythroid cell, when measured at a similar timepoint after introduction of the mRNA. In embodiments, the timepoint is 1, 2, 3, 4, 5, 6, 7, 14, 21, or 28 days after the cell is contacted with the mRNA.

In embodiments, a cell described herein comprises a heterologous UTR, e.g., a heterologous UTR comprising a regulatory element, and further comprises a chemical modification, e.g., comprises one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof.

In some embodiments, a contacting step described herein (e.g., contacting the erythroid cell with mRNA) occurs before enucleation of the cell, and in other embodiments, the contacting step occurs after enucleation of the cell. In embodiments, the contacting step is performed on a population of cells comprising a plurality of enucleated cells and a plurality of nucleated cells. The population of cells may be, e.g., primarily nucleated or primarily enucleated. In embodiments, the method comprises culturing the cells under conditions suitable for enucleation.

In some embodiments of any of the methods herein, providing comprises contacting an erythroid cell, e.g., a nucleated erythroid cell, with an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element, (e.g., isolated RNA or in vitro transcribed RNA). In embodiments, providing comprises receiving the erythroid cell from another entity.

In embodiments, the parameter described herein is selected from: the ability to express the exogenous protein; the structure or function of the exogenous protein; the proportion of cells comprising the endogenous mRNA; the proportion of cells comprising the endogenous protein; the level of exogenous mRNA in the cell; the level of exogenous protein in the cell; cell proliferation rate; or cell differentiation state. In embodiments, the method comprises comparing a value for the preselected parameter with a reference. In embodiments, the method comprises, responsive to the value for the parameter, or a comparison of the value with a reference, classifying, approving, or rejecting the cell or batch of cells.

In some embodiments, a cell described herein is disposed in a population of cells. In embodiments, the population of cells comprises a plurality of cells as described herein, and optionally further comprises one or more other cells, e.g., wild-type erythroid cells that lack the exogenous mRNA, nucleated erythroid cells, or non-erythroid cells. In embodiments, the population of cells comprises at least a first cell comprising a first exogenous RNA and a second cell comprising a second exogenous RNA. In embodiments, the population of cells comprises at least a first cell comprising a first exogenous RNA and a second exogenous RNA.

In embodiments, the RNA is produced by in vitro transcription or solid phase chemical synthesis.

In some embodiments, e.g., in embodiments involving contacting erythroid cells with an exogenous mRNA during maturation phase, the contacting comprises electroporation. In some embodiments, the contacting is performed at between days 6-8, 5-9, 4-10, 3-11, 2-12, or 1-13 of maturation. In some embodiments, the contacting is performed on or after day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 of maturation. In some embodiments, the method further comprises culturing the cells for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 days after the contacting. In some embodiments, the method further comprises testing expression of the transgenic mRNA, e.g., detecting a level of a protein encoded by the transgenic mRNA, after the contacting, e.g., at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days after contacting the cell with the mRNA.

In some aspects, the disclosure provides a method of making an erythroid cell comprising an mRNA encoding an exogenous protein, comprising:

a) providing an erythroid cell in maturation phase, and

b) contacting (e.g., electroporating) the erythroid cell with an mRNA encoding the exogenous protein, under conditions that allow uptake of the mRNA by the erythroid cell,

thereby making an erythroid cell comprising an mRNA encoding an exogenous protein.

In embodiments, the method comprises providing a population of erythroid cells in maturation phase and contacting the population with the mRNA encoding the exogenous protein. In embodiments, a plurality of erythroid cells of the population each takes up an mRNA encoding the exogenous protein. In embodiments the cell expresses the exogenous protein. In embodiments the cell comprises the exogenous protein. In embodiments, a plurality of cells in the population express the exogenous protein. In embodiments, the population of cells in maturation phase is a population of cells expanded in a maturation medium for 3-7 days, e.g., 4-5 or 4-6 days. In embodiments, the population of cells in maturation phase is a population described herein, e.g., having a specified percent enucleation, translational activity, or cell surface marker expression.

In embodiments, at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells in the population comprise the exogenous protein, e.g., 5 days after contacting with the mRNA. In embodiments, the cells in the population comprise at least 1,000, 2,000, 5,000, 10,000, 20,000,

50,000, or 100,000 copies of the exogenous protein, e.g., 5 days after the contacting with the mRNA. In embodiments, the cells comprise at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the exogenous protein for at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days after contacting with the mRNA. In embodiments, the cells comprise at least 1,000 copies of the exogenous protein for at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days after contacting with the mRNA.

In some aspects, the disclosure provides a method of making an erythroid cell comprising an mRNA encoding an exogenous protein, comprising:

a) providing an erythroid cell in maturation phase, and

b) contacting the erythroid cell with an mRNA encoding the exogenous protein, under conditions that allow uptake of the mRNA by the erythroid cell,

thereby making an erythroid cell comprising an mRNA encoding an exogenous protein.

In embodiments, the method comprises providing a population of erythroid cells in maturation phase and contacting a plurality of cells of the population of erythroid cells with the mRNA encoding the exogenous protein. In embodiments, the population of erythroid cells in maturation phase is a population of cells expanded in a maturation medium for 3-7 days, e.g., 4-5 or 4-6 days. In embodiments, the population of erythroid cells is a population of erythroid cells comprising one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or more) of the following properties:

i.a) 2-40%, 3-33%, 5-30%, 10-25%, or 15-20% of the cells in the population are enucleated;

i.b) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 2%, 3%, 4%, or 5% of the cells in the population are enucleated;

i.c) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 6%, 10%, 15%, 20%, or 25% of the cells in the population are enucleated;

i.d) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 30%, 35%, 40%, 45%, or 50% of the cells in the population are enucleated;

i.e) no more than 1%, 2%, 3%, 5%, 10%, 15%, or 20% of the cells in the population are enucleated;

i.f) no more than 25%, 30%, 35%, 40%, 45%, or 50% of the cells in the population are enucleated;

i.g) the population of cells has reached 6-70%, 10-60%, 20-50%, or 30-40% of maximal enucleation;

i.h) the population of cells has reached no more than 1%, 2%, 3%, 5%, 10%, 15%, or 20% of maximal enucleation;

i.i) the population of cells has reached no more than 25%, 30%, 35%, 40%, 45%, 50%, or 60% of maximal enucleation;

ii.a) the population of cells is fewer than 3, 2, or 1 population doubling from a plateau in cell division;

ii.b) the population of cells is capable of fewer than 3, 2, or 1 population doubling;

ii.c) the population will increase by no more than 1.5, 2, or 3 fold before the population reaches an enucleation level of at least 70% of cells in the population;

iii.a) at least 80%, 85%, 90%, 95%, or 99% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.b) at least 50%, 60%, 70%, 75%, or 79% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.c) 30-90%, 40-90%, 50-90%, 60-90%, or 70-90% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.d) at least 80%, 85%, 90%, 95%, or 99% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast);

iii.e) at least 50%, 60%, 70%, 75%, or 79% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast); or

iii.f) 30-90%, 40-90%, 50-90%, 60-90%, or 70-90% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast).

In embodiments, prior to or after contacting the plurality of cells with the mRNA encoding the exogenous protein, the plurality of cells are separated from the population of erythroid cells, e.g., the plurality of cells are separated from the population based on enucleation status (e.g., the plurality of cells are nucleated cells and the rest of the population are enucleated cells).

In embodiments, prior to or after contacting the plurality of cells with the mRNA encoding the exogenous protein, the method further comprises synchronizing the differentiation stage of the population of erythroid cells, e.g., by arresting the growth, development, hemoglobin

synthesis, or the process of enucleation of the population, e.g., by incubating the population with an inhibitor of enucleation (e.g., an inhibitor of histone deacetylase (HDAC), an inhibitor of mitogen-activated protein kinase (MAPK), an inhibitor of cyclin-dependent kinase (CDK), or a proteasome inhibitor). In embodiments, arresting occurs prior to enucleation of more than 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10% of the cells in the population.

In some aspects, the disclosure provides a method of manufacturing a population of reticulocytes that express an exogenous protein, the method comprising:

(a) providing a population of erythroid precursor cells (e.g., CD34+ cells);

(b) culturing the population of erythroid precursor cells under differentiating conditions to provide a population of differentiating erythroid cells;

(c) contacting the differentiating erythroid cells with an mRNA encoding the exogenous protein, under conditions that allow uptake of the mRNA by the differentiating erythroid cells, wherein the contacting is performed when the population of differentiating erythroid cells is between 0.1 and 25% enucleated (e.g., between 0.1 and 20% enucleated, between 0.1 and 15% enucleated, between 0.1 and 12% enucleated, or between 0.1 and 10% enucleated); and

(d) further culturing the differentiating erythroid cells to provide a population of reticulocytes,

thereby manufacturing a population of reticulocytes that express the exogenous protein.

In embodiments, the further culturing comprises fewer than 3, 2, or 1 population doubling. In embodiments, the contacting is performed when at least 50% (at least 60%, 70%, 75%, 80%, 90%, or 95%) of the differentiating erythroid cells exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast).

In some aspects, the disclosure provides a method of manufacturing a population of reticulocytes that express an exogenous protein, comprising (a) providing a population of erythroid precursor cells, (b) culturing the population of erythroid precursor cells under differentiating conditions to provide a population of differentiating erythroid cells, (c) contacting the differentiating erythroid cells with an mRNA encoding the exogenous protein, wherein the improvement comprises: the contacting is performed when the population of differentiating erythroid cells is between 0.1 and 25% enucleated (e.g., between 0.1 and 20% enucleated,

between 0.1 and 15% enucleated, between 0.1 and 12% enucleated, or between 0.1 and 10% enucleated).

In embodiments, the contacting is performed when the population of differentiating erythroid cells has fewer than 3, 2, or 1 population doubling before a plateau in cell division. In embodiments, the contacting is performed when at least 50% (at least 60%, 70%, 75%, 80%, 90%, or 95%) of the differentiating erythroid cells exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast).

In some aspects, the disclosure provides a method of making an erythroid cell comprising an mRNA that encodes an exogenous protein, comprising:

providing a reaction mixture comprising an erythroid cell and an mRNA encoding the exogenous protein, under conditions which inhibit degradation of mRNA, e.g., by inclusion in the reaction mixture a ribonuclease inhibitor, and

maintaining the reaction mixture under conditions that allow uptake of the mRNA by the erythroid cell,

thereby making an erythroid cell comprising an mRNA encoding an exogenous protein.

In embodiments, the method comprises providing a population of erythroid cells and contacting the population with the mRNA encoding the exogenous protein. In embodiments, a plurality of erythroid cells of the population each takes up an mRNA encoding the exogenous protein. In embodiments, the cell or plurality of cells express the exogenous protein. In embodiments, the cell or plurality of cells comprises the exogenous protein. In embodiments, the method further comprises electroporating the cell or population of cells. In embodiments, the method further comprises contacting a population of erythroid cells with a ribonuclease inhibitor. In embodiments, the method comprises contacting the population of cells with the ribonuclease inhibitor before, during, or after contacting the cells with the mRNA. In embodiments, the method comprises contacting the cells with the ribonuclease inhibitor at day 4, 5, or 6 of maturation phase. In embodiments, the cell is in maturation phase. In embodiments, the population of cells in maturation phase is a population described herein, e.g., having a specified percent enucleation, translational activity, or cell surface marker expression.

In embodiments, the mRNA is in vitro transcribed mRNA.

In embodiments, at least 80%, 85%, 90%, or 95% (and optionally up to 95%) of the cells of the population are viable (e.g., as determined by Annexin V staining) 5 days after the cells are contacted with the mRNA. In embodiments, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the cells of the population are enucleated 5 days after the cells are contacted with the mRNA. In embodiments, the proportion of cells that are enucleated 5 days after the cells are contacted with the mRNA is at least 50%, 60%, 70%, 80%, 90%, or 95% of the proportion of cells that are enucleated in an otherwise similar population of cells not treated with the ribonuclease inhibitor. In embodiments, the population of cells comprises at least 1 x 10^6, 2 x 10^6, 5 x 10^6, 1 x 10^7, 2 x 10^7, 5 x 10^7, or 1 x 10^8 cells at the time the cells are contacted with the mRNA. In embodiments, the population of cells expands by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% within 5 days after the cells are contacted with the mRNA. In embodiments, at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population express the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA. In embodiments, at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population comprise at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA. In embodiments, at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population comprise at least 1,000 copies of the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA. In embodiments, at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population comprise at least 10,000 copies of the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA. In embodiments, the population of cells comprises at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% more, or at least 2-fold, 3-fold, 4-fold, or 5-fold more of the exogenous protein than an otherwise similar population of cells not treated with the ribonuclease inhibitor.

The disclosure also provides, in some aspects, a reaction mixture comprising: i) an erythroid cell, ii) an mRNA comprising an exogenous protein and iii) a ribonuclease inhibitor.

In embodiments, the mRNA is inside the erythroid cell. In embodiments, the reaction mixture comprises a plurality of erythroid cells.

The disclosure also provides, in some aspects, a method of assaying a reaction mixture comprising enucleated erythroid cells that comprise an exogenous protein for a ribonuclease inhibitor, comprising:

providing a reaction mixture comprising enucleated erythroid cells that comprise an exogenous protein,

assaying for the presence or level of a ribonuclease inhibitor, e.g., by ELISA, Western blot, or mass spectrometry, e.g., in an aliquot of the reaction mixture.

In embodiments, the method comprises comparing the level of ribonuclease inhibitor to a reference value.

In embodiments, the method further comprises, responsive to the comparison, performing one or more of:

classifying the population, e.g., as meeting a requirement or not meeting a requirement, e.g., wherein the requirement is met when the level of ribonuclease inhibitor is below the reference value,

classifying the population as suitable or not suitable for a subsequent processing step, e.g., when the population is suitable for a subsequent purification step when the level of ribonuclease inhibitor is above the reference value,

classifying the population as suitable or not suitable for use as a therapeutic, or

formulating or packaging the population, or an aliquot thereof, for therapeutic use, e.g., when the level of ribonuclease inhibitor is below the reference value.

In embodiments, the ribonuclease inhibitor is RNAsin Plus (e.g., from Promega), Protector RNAse Inhibitor (e.g., from Sigma), or Ribonuclease Inhibitor Huma (e.g., from Sigma).

The disclosure also provides, in some aspects, a method of making an erythroid cell comprising an mRNA that encodes an exogenous protein, comprising:

providing a reaction mixture comprising an erythroid cell and an mRNA encoding the exogenous protein, under conditions which inhibit protein degradation, e.g., by inclusion in the reaction mixture a protease inhibitor, e.g., a proteasome inhibitor, and

maintaining the reaction mixture under conditions that allow uptake of the mRNA by the erythroid cell,

thereby making an erythroid cell comprising an mRNA encoding an exogenous protein.

In embodiments, the method comprises providing a population of erythroid cells and contacting the population with the mRNA encoding the exogenous protein. In embodiments, a plurality of erythroid cells of the population each takes up an mRNA encoding the exogenous protein. In embodiments, the cell or plurality of cells express the exogenous protein. In embodiments, the cell or plurality of cells comprises the exogenous protein. In embodiments, the method further comprises electroporating the cell or population of cells. In embodiments, the method further comprises contacting the population of erythroid cells with a proteasome inhibitor. In embodiments, the method comprises contacting the population of cells with the proteasome inhibitor before, during, or after contacting the cells with the mRNA, e.g., 0.5-2 days before or after contacting the cells with the mRNA. In embodiments, the method comprises contacting the population of cells with the proteasome inhibitor 0.5-2 days before contacting the cells with the mRNA. In embodiments, the method comprises removing the proteasome inhibitor (e.g., by washing the cells) before electroporation.

In embodiments, the method comprises contacting the cells with the proteasome inhibitor at day 3-7 of maturation, e.g., day 4, 5, or 6 of maturation phase. In embodiments, the cell is in maturation phase. In embodiments, the population of cells in maturation phase is a population described herein, e.g., having a specified percent enucleation, translational activity, or cell surface marker expression.

In embodiments, the mRNA is in vitro transcribed mRNA. In embodiments, at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population are viable 5 days after the cells are contacted with the mRNA. In embodiments, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the cells of the population are enucleated 5 days after the cells are contacted with the mRNA. In embodiments, the proportion of cells that are enucleated 5 days after the cells are contacted with the mRNA is at least 50%, 60%, 70%, 80%, 90%, or 95% of the proportion of cells that are enucleated in an otherwise similar population of cells not treated with the proteasome inhibitor. In embodiments, the population of cells comprises at least 1×10^6, 2×10^6, 5×10^6, 1×10^7, 2×10^7, 5×10^7, or 1×10^8 cells at the time the cells are contacted with the mRNA.

In embodiments, the population of cells expands by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% within 5 days after the cells are contacted with the mRNA. In embodiments,

at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population express the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA. In embodiments, at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population comprise at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA. In embodiments, at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population comprise at least 1,000 copies of the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA. In embodiments, at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population comprise at least 10,000 copies of the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA. In embodiments, the population of cells comprises at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% more, or at least 2-fold, 3-fold, 4-fold, or 5-fold more of the exogenous protein than an otherwise similar population of cells not treated with the proteasome inhibitor.

In some aspects, the disclosure provides a reaction mixture comprising: i) an erythroid cell, ii) an mRNA comprising an exogenous protein and iii) a proteasome inhibitor.

In embodiments, the mRNA is inside the erythroid cell. In embodiments, the reaction mixture comprises a plurality of erythroid cells.

The disclosure also provides, in some aspects method of assaying a reaction mixture comprising enucleated erythroid cells that comprise an exogenous protein for a proteasome inhibitor, comprising:

providing a reaction mixture comprising enucleated erythroid cells that comprise an exogenous protein,

assaying for the presence or level of a proteasome inhibitor, e.g., by ELISA, Western blot, or mass spectrometry, e.g., in an aliquot of the reaction mixture.

In embodiments, the method further comprises comparing the level of proteasome inhibitor to a reference value.

In embodiments, the method further comprises, responsive to the comparison, one or more of:

classifying the population, e.g., as meeting a requirement or not meeting a requirement, e.g., wherein the requirement is met when the level of proteasome inhibitor is below the reference value,

classifying the population as suitable or not suitable for a subsequent processing step, e.g., when the population is suitable for a subsequent purification step when the level of proteasome inhibitor is above the reference value,

classifying the population as suitable or not suitable for use as a therapeutic,

formulating or packaging the population, or an aliquot thereof, for therapeutic use, e.g., when the level of proteasome inhibitor is below the reference value.

In embodiments, the proteasome inhibitor is a 20S proteasome inhibitor, e.g., MG-132 or carfilzomib, or a 26S proteasome inhibitor, e.g., bortezomib.

In embodiments, the method of making an erythroid cell comprising an mRNA encoding a first exogenous protein and a second exogenous protein, comprising:

a) providing an erythroid cell, e.g., in maturation phase, and

b) contacting the erythroid cell with an mRNA encoding the first exogenous protein and a second mRNA encoding the second exogenous protein, under conditions that allow uptake of the first mRNA and second mRNA by the erythroid cell,

thereby making an erythroid cell comprising the first mRNA and the second mRNA.

In embodiments, the erythroid cell comprises at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the first exogenous protein and the second exogenous protein, e.g., 5 days after the contacting with the mRNA.

The disclosure also provides, in some aspects, a method of producing a population of erythroid cells expressing a first exogenous protein and a second exogenous protein, comprising:

a) providing a population of erythroid cells, e.g., in maturation phase, and

b) contacting the population of erythroid cells with a first mRNA encoding a first protein and a second mRNA encoding a second protein,

thereby making an erythroid cell comprising an mRNA encoding an exogenous protein

wherein at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of cells in the population comprise both of the first mRNA and the second mRNA.

In embodiments, the population of erythroid cells comprises an average of at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the first exogenous protein and the second exogenous protein per cell, e.g., 5 days after the contacting with the mRNA.

In embodiments, the contacting comprises performing electroporation.

In embodiments, the population of cells comprises the first exogenous protein and the second exogenous protein in at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of cells for at least 5 days after the cells were contacted with the first and second mRNAs. In embodiments, the population of cells comprises the first exogenous protein and the second exogenous protein in at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of cells for at least 2, 4, 6, 8, 10, 12, or 14 days after the cells were contacted with the first and second mRNAs. In embodiments, the population of cells comprises the first exogenous protein and the second exogenous protein in at least 80% of cells for at least 2, 4, 6, 8, 10, 12, or 14 days after the cells were contacted with the first and second mRNAs.

In embodiments, the first exogenous protein has an amino acid length that is no more than 10%, 20%, 30%, 40%, or 50% longer than that of the second exogenous protein. In some embodiments, the average level of the second exogenous protein is no more than 10%, 20%, 30%, 40%, or 50% of the level of the first exogenous protein in the erythroid cell population.

In embodiments, the first exogenous protein has an amino acid length that is at least 50%, 60%, 70%, 80%, 90%, 2-fold, or 3-fold longer than that of the second exogenous protein. . In some embodiments, the average level of the second exogenous protein is at least 50%, 60%, 70%, 80%, 90%, 2-fold, or 3-fold higher than the level of the first exogenous protein in the erythroid cell population.

The disclosure also provides, in certain aspects, a population of erythroid cells wherein at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of cells in the population express a first exogenous protein and a second exogenous protein, wherein the population was not made by contacting the cells with DNA encoding the first or second exogenous protein.

The disclosure also provides, in certain aspects, method of producing a plurality of erythroid cells comprising a predetermined number of copies of an exogenous protein per cell, comprising contacting the population with a predetermined amount of mRNA encoding the exogenous protein, thereby making the erythroid cell comprising the predetermined amount of the exogenous protein. In embodiments, the method further comprises evaluating one or more of the plurality of erythroid cells (e.g., enucleated erythroid cells) to determine the amount of the exogenous protein.

In some aspects, the disclosure provides a method of evaluating the amount of an exogenous protein in a sample of erythroid cells, e.g., enucleated erythroid cells comprising:

providing a plurality of erythroid cells comprising a predetermined number of copies of an exogenous protein per cell, which was made by contacting the population with a predetermined amount of mRNA encoding the exogenous protein, and

determining the amount of the exogenous protein in the plurality of erythroid cells.

In some embodiments, the method comprises:

contacting the cell population with 0.6 ±50%, ±20% or ±10% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 1,000,000±50%, ±20% or ±10% copies of the exogenous protein per cell,

contacting the cell population with 0.4±50%, ±20% or ±10% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 870,000 ±50%, ±20% copies of the exogenous protein per cell,

contacting the cell population with 0.2 ±50%, ±20% or ±10% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 610,000 ±50%, ±20%, or ±10% copies of the exogenous protein per cell,

contacting the cell population with 0.1 ±50%, ±20% or ±10% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 270,000 ±50%, ±20%, or ±10% copies of the exogenous protein per cell,

contacting the cell population with 0.05 ±50%, ±20% or ±10% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 100,000 ±50%, ±20%, or ±10% copies of the exogenous protein per cell, or

contacting the cell population with 0.025 ±50%, ±20% or ±10% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 43,000 ±50%, ±20%, or ±10% copies of the exogenous protein per cell.

In embodiments, at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population express the exogenous protein 1 day after the cells are contacted with the exogenous protein.

In some embodiments of any of the aspects herein, the population of erythroid cells (e.g., the population of cells that is contacted with an mRNA) as described herein is a population of erythroid cells wherein one or more (e.g., 2, 3, 4, 5, 6, 7, 8 or more) of:

2-40%, 3-33%, 5-30%, 10-25%, or 15-20% of the cells in the population are enucleated;

less than 3%, 5%, 10%, 20%, or 30% of the cells in the population are enucleated;

greater than 0 (e.g., 0.1%, 0.2%, 0,5%) and no more than 50% (40%, 30%, 20%, 18%, 15%, 12%, 10%) of the cells in the population are enucleated;

the population of cells has reached 6-70%, 10-60%, 20-50%, or 30-40% of maximal enucleation;

the population of cells has reached less than 6%, 10%, 20%, 30%, 40%, 50%, or 60% of maximal enucleation;

the population of cells has a translational activity of at least 600,000, 800,000, 1,000, 000, 1,200,000, 1,400,000, 1,600,000, 1,800,000, 2,000,000, 2,200,000, or 2,400,000 as measured by a BONCAT assay, e.g., by the translation assay of Example 10;

the population of cells has a translational activity of 600,000-2,400,000, 800,000-2,200,000, 1,000, 000-2,000,000, 1,200,000-1,800,000, or 1,400,000-1,600,000 as measured by a BONCAT assay, e.g., by the translation assay of Example 10;

the population of cells in maturation phase has at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of maximal translational activity, wherein maximal translational activity refers to the maximal translational activity of a similar number of precursors or progenitors of the cells in maturation phase, e.g., CD34+ cells;

between 0.1-25% of the cells in the population are enucleated and the population of cells is fewer than 1, 2 or 3 population doublings from a plateau in cell division;

the population of cells is fewer than 3, 2, or 1 population doubling from a plateau in cell division;

the population of cells is capable of fewer than 3, 2, or 1 population doubling;

the population will increase by no more than 1.5, 2, or 3 fold before the population reaches an enucleation level of at least 70% of cells in the population;

84-99%, 85-95%, or about 90% of the cells in the population are GPA-positive (e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10);

at least 84%, 85%, 90%, 95%, or 99% of the cells in the population are GPA-positive (e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10);

54-99%, 55-98%, 60-95%, 65-90%, 70-85%, or 75-80% of the cells in the population are band3-positive (e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10);

at least 54%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% of the cells in the population are band3-positive (e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10);

96-100%, 97-99%, or about 98% of the cells in the population are alpha4 integrin-positive (e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10);

at least 95%, 96%, 97%, 98%, or 99% of the cells in the population are alpha4 integrin-positive (e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10);

at least 50% (e.g., at least 60%, 70%, 80%, 85%, 90%, 92%, 94%, 96%) of the cells in the population are alpha4 integrin-positive and band3-positive; or

at least 50% of the cells in the population are band3-positive and at least 90%-95% are alpha4 integrin-positive.

The disclosure contemplates all combinations of any one or more of the foregoing aspects and/or embodiments, as well as combinations with any one or more of the embodiments set forth in the detailed description and examples.

Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references (e.g., sequence database reference numbers) mentioned herein are incorporated by reference in their entirety. For example, all GenBank, Unigene, and Entrez sequences referred to herein, e.g., in any Table herein, are incorporated by reference. Unless otherwise specified, the sequence accession numbers specified herein, including in any Table herein, refer to the database entries current as of July 7, 2016. When one gene or protein references a plurality of sequence accession numbers, all of the sequence variants are encompassed.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a plot showing expression of various constructs of differing sizes on K562 erythroleukemia cells following lentiviral transduction. Each data point represents a unique construct. Expression is measured by flow cytometry with an anti-HA antibody, as every

construct contains the appropriate epitope tag. The constructs are arrayed by provirus length, which is the length of nucleic acid in the viral genome (including the transgene itself) that will be integrated into the target cell genome.

Fig. 2 is a plot showing a characterization of lentivirus particles that contain transgenes of various lengths such that the provirus ranges from approximately 3.5 kb to approximately 8.5 kb. The y-axis shows RNA copies per ug of p24. The number of RNA copies per mL of viral supernatant is measured by qPCR. The amount of p24 (ug) per mL of viral supernatant is measured by ELISA against p24. The ratio of the two measured values gives the number of RNA copies per mass p24.

Fig. 3 shows flow cytometry histograms showing the expression of GFP in K562 cells and erythroid cells cultured from primary progenitors as measured by flow cytometry 24 hrs following electroporation of cells with GFP mRNA using conditions optimized for K562 cells.

Fig. 4A, Fig. 4B, and Fig. 4C are flow cytometry histograms showing the expression of GFP in erythroid cells cultured from primary progenitors as measured by flow cytometry 24 hrs following electroporation of cells with GFP mRNA. 12 different conditions are shown (numbers 1-12). In the first column, GFP fluorescence is detected. In the second column, cell viability is measured with Life Technologies LIVE/DEAD stain, wherein the dead cells are stained by the dye, such that the percentage of live cells is 100% - %Fluorescent Cells.

Fig. 5 shows flow cytometry histograms showing expression of GFP in erythroid cells cultured from primary progenitors at various stages of differentiation as measured by flow cytometry 24 hrs following electroporation of cells with GFP mRNA. Untransfected cells are compared to GFP mRNA transfected cells. The columns refer to the number of days of erythroid differentiation prior to transfection. The percent viability is measured with Life Technologies LIVE/DEAD stain and is reported as the % of viable cells, that is, cells that stain negative for the dye.

Fig. 6 shows flow cytometry histograms showing the expression of GFP in erythroid cells cultured from primary progenitors as measured by flow cytometry 24 hrs following electroporation of cells with GFP mRNA. Cells were transfected at day 9 of culture then returned to differentiation media and re-analyzed at day 13. At day 13, cells were re-electroporated with GFP mRNA and analyzed for expression 24 hrs later.

Fig.7A, 7B, and 7C show the percent of GFP positive erythroid cells electroporated at different timepoints after the start of in vitro differentiation. Fig. 7A illustrates the expansion, differentiation, and maturation phases. Fig. 7B shows the percentage of GFP positive cells after electroporation on differentiation day 9, when assayed through maturation day 9. Fig. 7C shows the percentage of GFP positive cells after electroporation on maturation day 7, when assayed through maturation day 16. "No EP" denotes the no-electroporation control. "P1-P4" denote four electroporation conditions.

Fig. 8A and 8B are graph showing GFP expression in erythroid cells expressing GFP at the indicated timepoints, when the erythroid cells were electroporated with mRNA encoding GFP on days M4 through M7 of maturation. **Fig. 8A** shows the percentage of cells expressing GFP, and **Fig. 8B** shows the mean fluorescent intensity of the cells.

Fig. 9 is a graph showing a time course of erythroid cell maturation. Circles indicate levels of translation, measured by AHA intensity/incorporation. Squares indicate enucleation levels.

Fig. 10 is a graph showing a time course of erythroid cell maturation, where the percentage of cells expressing mCherry is shown on the y-axis. EP, electroporated control (without RNasin). UT no EP, untransfected control, no electroporation. EP + RNasin 0.5, electroporated sample treated with 0.5 U/uL RNasin. EP + RNasin 1, electroporated sample treated with 1 U/uL RNasin. EP + RNasin 2, electroporated sample treated with 2 U/uL RNasin.

Fig. 11 is a graph showing effective expression (mean fluorescent intensity x number of fluorescent cells)/1x10^6) versus time of cells treated with proteasome inhibitors at different timepoints.

Fig. 12 is a graph showing percentage of GFP-positive cells for cells electrporated with GFP-PAL naked mRNA, GFP-PAL polyA Cap mRNA, GFP-PAL naked modified mRNA, or GFP-PAL polyA Cap modified mRNA at day M4. GFP expression was measured by flow cytometry at days M5 (24 hours later), M6, M7, and M10.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, the term "antibody molecule" refers to a protein, e.g., an immunoglobulin chain or fragment thereof, comprising at least one immunoglobulin variable domain sequence.

The term "antibody molecule" encompasses antibodies and antibody fragments. In an embodiment, an antibody molecule is a multispecific antibody molecule, e.g., a bispecific antibody molecule. Examples of antibody molecules include, but are not limited to, Fab, Fab', F(ab')2, Fv fragments, scFv antibody fragments, disulfide-linked Fvs (sdFv), a Fd fragment consisting of the VH and CH1 domains, linear antibodies, single domain antibodies such as sdAb (either VL or VH), camelid VHH domains, multi-specific antibodies formed from antibody fragments such as a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, an isolated epitope binding fragment of an antibody, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv.

As used herein, "differentiating conditions" are conditions under which an erythroid precursor cell, e.g., an HSC, or CD34+ cell, is amplified and differentiated into an enucleated erythroid cell (e.g., an enucleated reticulocyte or erythrocyte) in ex-vivo culture, typically with the addition of erythropoietin and other growth factors. This process typically includes a proliferation/expansion phase, a differentiation phase, and a maturation phase (during which the cells lose their nuclei). Differentiating conditions are known in the art. See for example, Olivier et al., *Novel, High-Yield Red Blood Cell Production Methods from CD34-Positive Cells Derived from Human Embryonic Stem, Yolk Sac, Fetal Liver, Cord Blood, and Peripheral Blood.* Stem Cells Transl Med. 2012 Aug; 1(8): 604–614, and references cited therein. "Erythroid cells" as used herein are cells of the erythrocytic series including erythroid precursor cells such as hematopoietic stem cells (HSCs) and nucleated erythroid precursor cells such as CD34+ cells, nucleated red blood cell precursors, enucleated red blood cells (e.g., reticulocytes or erythrocytes), and any intermediates between erythroid precursor cells and enucleated erythrocytes. In an embodiment, an erythroid cell is a proerythroblast, basophilic erythroblast, polychromatophilic erythroblast, orthochromatic erythroblast, reticulocyte, or erythrocyte. In an embodiment, an erythroid cell is a cord blood stem cell, a CD34+ cell, a hematopoietic stem cell (HSC), a spleen colony forming (CFU-S) cell, a common myeloid progenitor (CMP) cell, a blastocyte colony-forming cell, a burst forming unit-erythroid (BFU-E), a megakaryocyte-erythroid progenitor (MEP) cell, an erythroid colony-forming unit (CFU-E), a reticulocyte, an erythrocyte, an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), a polychromatic normoblast, an orthochromatic normoblast, or a combination thereof.

In embodiments, the erythroid cells are, or are derived from, immortal or immortalized cells. For example, immortalized erythroblast cells can be generated by retroviral transduction of CD34+ hematopoietic progenitor cells to express Oct4, Sox2, Klf4, cMyc, and suppress TP53 (e.g., as described in Huang et al. (2013) Mol Ther, epub ahead of print September 3). In addition, the cells may be intended for autologous use or provide a source for allogeneic transfusion. In some embodiments, erythroid cells are cultured.

As used herein, "enucleated" refers to a cell that lacks a nucleus, e.g., a cell that lost its nucleus through differentiation into a mature red blood cell.

"Exogenous polypeptide" refers to a polypeptide that is not produced by a wild-type cell of that type or is present at a lower level in a wild-type cell than in a cell containing the exogenous polypeptide. In some embodiments, an exogenous polypeptide is a polypeptide encoded by a nucleic acid that was introduced into the cell, which nucleic acid is optionally not retained by the cell.

"Exogenous" when used to modify the term mRNA, refers to the relationship between the mRNA and a selected subject cell, e.g., an erythroid cell, e.g., an enucleated erythroid cell. An exogenous mRNA does not exist naturally in the subject cell. In an embodiment an exogenous mRNA expresses a polypeptide that does not occur naturally in the selected subject cell (an exogenous polypeptide). In embodiments an exogenous mRNA comprises a first portion that does not occur naturally in the selected subject cell and a second portion that does occur naturally in the selected subject cell.

"Heterologous" when used to modify the term untranslated region (UTR), refers to the relationship between the UTR and a coding region with which the UTR is operatively linked (the subject coding region). A UTR is a heterologous UTR if it has one or more of the following properties: i) it does not exist in nature; ii) it does not occur naturally with the subject coding region, e.g., differs by at least 1 nucleotide, e.g., by at least 1, 2, 3, 4, 5, 10, 20, 25, 30, 35, 40, 45, or 50 % of its nucleotides, from the UTR which occurs naturally operatively linked with the subject coding region; or iii) wherein the UTR does not occur naturally operatively linked to the subject coding region but occurs naturally operatively linked with a coding region other than the subject coding region, or has at least at least 70, 80, 90, 95, 99, or 100% homology to such naturally occurring UTR.

"Modified" as used herein in reference to a nucleic acid, refers to a structural characteristic of that nucleic acid that differs from a canonical nucleic acid. It does not imply any particular process of making the nucleic acid or nucleotide.

The term "regulatory element", as used herein in reference to an RNA sequence, refers to a sequence that is capable of modulating (e.g., upregulating or downregulating) a property of the RNA (e.g., stability or translatability, e.g., translation level of the coding region to which the regulatory element is operatively linked) in response to the presence or level of a molecule, e.g., a small molecule, RNA binding protein, or regulatory RNA such as a miRNA.

Chemically modified nucleic acids

The exogenous RNA can comprise unmodified or modified nucleobases. Naturally occurring RNAs are synthesized from four basic ribonucleotides: ATP, CTP, UTP and GTP, but may contain post-transcriptionally modified nucleotides. Further, approximately one hundred different nucleoside modifications have been identified in RNA (Rozenski, J, Crain, P, and McCloskey, J. (1999). The RNA Modification Database: 1999 update. Nucl Acids Res 27: 196-197). An RNA can also comprise wholly synthetic nucleotides that do not occur in nature.

In some embodiments, the chemically modification is one provided in PCT/US2016/032454, US Pat. Pub. No. 20090286852, of International Application No. WO/2012/019168, WO/2012/045075, WO/2012/135805, WO/2012/158736, WO/2013/039857, WO/2013/039861, WO/2013/052523, WO/2013/090648, WO/2013/096709, WO/2013/101690, WO/2013/106496, WO/2013/130161, WO/2013/151669, WO/2013/151736, WO/2013/151672, WO/2013/151664, WO/2013/151665, WO/2013/151668, WO/2013/151671, WO/2013/151667, WO/2013/151670, WO/2013/151666, WO/2013/151663, WO/2014/028429, WO/2014/081507, WO/2014/093924, WO/2014/093574, WO/2014/113089, WO/2014/144711, WO/2014/144767, WO/2014/144039, WO/2014/152540, WO/2014/152030, WO/2014/152031, WO/2014/152027, WO/2014/152211, WO/2014/158795, WO/2014/159813, WO/2014/164253, WO/2015/006747, WO/2015/034928, WO/2015/034925, WO/2015/038892, WO/2015/048744, WO/2015/051214, WO/2015/051173, WO/2015/051169, WO/2015/058069, WO/2015/085318, WO/2015/089511, WO/2015/105926, WO/2015/164674, WO/2015/196130, WO/2015/196128, WO/2015/196118, WO/2016/011226, WO/2016/011222, WO/2016/011306, WO/2016/014846, WO/2016/022914, WO/2016/036902, WO/2016/077125, WO/2016/077123, each of which is herein incorporated by

reference in its entirety. It is understood that incorporation of a chemically modified nucleotide into a polynucleotide can result in the modification being incorporated into a nucleobase, the backbone, or both, depending on the location of the modification in the nucleotide. In some embodiments, the backbone modification is one provided in EP 2813570, which is herein incorporated by reference in its entirety. In some embodiments, the modified cap is one provided in US Pat. Pub. No. 20050287539, which is herein incorporated by reference in its entirety.

In some embodiments, the modified mRNA comprises one or more of ARCA: anti-reverse cap analog (m27.3'-OGP3G), GP3G (Unmethylated Cap Analog), m7GP3G (Monomethylated Cap Analog), m32.2.7GP3G (Trimethylated Cap Analog), m5CTP (5'-methyl-cytidine triphosphate), m6ATP (N6-methyl-adenosine-5'-triphosphate), s2UTP (2-thio-uridine triphosphate), and Ψ (pseudouridine triphosphate). In embodiments, the modified mRNA comprises N6-methyladenosine. In embodiments, the modified mRNA comprises pseudouridine.

In some embodiments, the exogenous RNA comprises a backbone modification, e.g., a modification to a sugar or phosphate group in the backbone. In some embodiments, the exogenous RNA comprises a nucleobase modification.

In some embodiments, the exogenous mRNA comprises one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3. For instance, in some embodiments, the exogenous mRNA comprises two or more (e.g., 3, 4, 5, 6, 7, 8, 9, or 10 or more) different types of chemical modifications. As an example, the exogenous mRNA may comprise two or more (e.g., 3, 4, 5, 6, 7, 8, 9, or 10 or more) different types of modified nucleobases, e.g., as described herein, e.g., in Table 1. Alternatively or in combination, the exogenous mRNA may comprise two or more (e.g., 3, 4, 5, 6, 7, 8, 9, or 10 or more) different types of backbone modifications, e.g., as described herein, e.g., in Table 2. Alternatively or in combination, the exogenous mRNA may comprise two or more (e.g., 3, 4, 5, 6, 7, 8, 9, or 10 or more) different types of modified cap, e.g., as described herein, e.g., in Table 3. For instance, in some embodiments, the exogenous mRNA comprises one or more type of modified nucleobase and one or more type of backbone modification; one or more type of modified nucleobase and one or more modified cap; one or more type of modified cap and one or more type of backbone modification; or one or more type

of modified nucleobase, one or more type of backbone modification, and one or more type of modified cap.

In some embodiments, the exogenous mRNA comprises one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, or more) modified nucleobases. In some embodiments, all nucleobases of the mRNA are modified. In some embodiments, the exogenous mRNA is modified at one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, or more) positions in the backbone. In some embodiments, all backbone positions of the mRNA are modified.

Heterologous untranslated regions

The exogenous mRNAs described herein can comprise one or more (e.g., two, three, four, or more) heterologous UTRs. The UTR may be, e.g., a 3' UTR or 5' UTR. In embodiments, the heterologous UTR comprises a eukaryotic, e.g., animal, e.g., mammalian, e.g., human UTR sequence, or a portion or variant of any of the foregoing. In embodiments, the heterologous UTR comprises a synthetic sequence. In embodiments, the heterologous UTR is other than a viral UTR, e.g., other than a hepatitis virus UTR, e.g., other than Woodchuck hepatitis virus UTR.

While not wishing to be bound by theory, in some embodiments, the 5' UTR is short, in order to reduce scanning time of the ribosome during translation. In embodiments, the untranslated region is less than 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 50, 40, 30, 20, 10, or 5 nucleotides in length. In embodiments, the 5'UTR comprises a sequence having not more than 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 50, 40, 30, 20, 10, or 5 consecutive nucleotides from a naturally occurring 5' UTR. In embodiments, the RNA lacks a 5' UTR.

In some embodiments, the 5' UTR does not comprise an AUG upstream of the start codon (uAUG). According to the non-limiting theory herein, some naturally occurring 5' UTRs contain one or more uAUGs which can regulate, e.g., reduce, translation of the encoded gene. Sometimes, the uAUGs are paired with stop codons, to form uORFs. Accordingly, in some embodiments, the 5' UTR has sequence similarity to a naturally occurring 5' UTR, but lacks one or more uAUGs or uORFs relative to the naturally occurring 5' UTR. The one or more uAUGs can be removed, e.g., by a deletion or substitution mutation.

It is understood that the heterologous UTRs provided herein can be provided as part of a purified RNA, e.g., by contacting an erythroid cell with an mRNA comprising the heterologous UTR. The heterologous UTRs herein can also be provided via DNA, e.g., by contacting the erythroid cell with DNA under conditions that allow the cell to transcribe the DNA into an RNA that comprises the heterologous UTR.

In embodiments, the 3' UTR comprises a polyA tail, e.g., at least 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, or 1000 adenosines.

In embodiments, the exogenous RNA comprises a 5' UTR and 3' UTR that allow circularization of the RNA through binding of an upstream element to a downstream element, directly or indirectly. In embodiments, the exogenous RNA comprises a 5' cap that participates in circularization.

UTRs comprising regulatory elements

In embodiments, the UTR comprises a regulatory element. The regulatory element may modulate (e.g., upregulate or downregulate) a property (e.g., stability or translation level) of the coding region to which it is operatively linked. In some embodiments, the regulatory element controls the timing of translation of the RNA. For instance, the RNA may be translated in response to phase of the cell cycle, presence or level of a pathogen (e.g., a virus that enters the cell), stage of red blood cell differentiation, presence or level of a molecule inside the cell (e.g., a metabolite, a signalling molecule, or an RNA such as a miRNA), or presence or level of a molecule outside the cell (e.g., a protein that is bound by a receptor on the surface of the red blood cell).

In embodiments, the regulatory element comprises a riboregulator, e.g., as described in Callura et al., "Tracking, tuning, and terminating microbial physiology using synthetic riboregulators" PNAS 107:36, p.15898-15903. In embodiments, the riboregulator comprises a hairpin that masks a ribosome binding site, thus repressing translation of the mRNA. In embodiments, a trans-activating RNA binds to and opens the hairpin, exposing the ribosome binding site, and allowing the mRNA to be translated. In embodiments, the ribosome binding site is an IRES, e.g., a Human IGF-II 5' UTR-derived IRES described in Pedersen, SK, et al., Biochem J. 2002 Apr 1; 363(Pt 1): 37–44:

GACCGGG CATTGCCCCC AGTCTCCCCC AAATTTGGGC ATTGTCCCCG GGTCTTCCAA CGGACTGGGC GTTGCTCCCG GACACTGAGG ACTGGCCCCG GGGTCTCGCT CACCTTCAGC AG (SEQ ID NO: 2)

In embodiments, the regulatory element comprises a toehold switch, e.g., as described in International Application WO2012058488. In embodiments, the toehold functions like a riboregulator and further comprises a short single stranded sequence called a toehold, which has homology to a trans-regulating RNA. In embodiments, the toehold can sample different binding partners, thereby more rapidly detecting whether the trans-regulating RNA is present.

In embodiments, the regulatory element is one described in Araujo et al., "Before It Gets Started: Regulating Translation at the 5′ UTR" Comparative and Functional Genomics, Volume 2012 (2012), Article ID 475731, 8 pages, which is herein incorporated by reference in its entirety.

In embodiments, the regulatory element comprises an upstream open reading frame (uORF). A uORF comprises a uAUG and a stop codon in-frame with the uAUG. uORFs often act as negative regulators of translation, when a ribosome translates the uORF and then stalls at the stop codon, without reaching the downstream coding region. An exemplary uORFs is that found in the fungal arginine attenuator peptide (AAP), which is regulated by arginine concentration. Another exemplary uORF is found in the yeast GCN4, where translation is activated under amino acid starvation conditions. Another uORF is found in Carnitine Palmitoyltransferase 1C (CPT1C) mRNA, where repression is relieved in response to glucose deprivation. In some embodiments, the uORF is a synthetic uORF. In some embodiments, the uORF is one found in the 5′ UTR of the mRNA for cyclin-dependent-kinase inhibitor protein (CDKN2A), thrombopoietin, hairless homolog, TGF-beta3, SRY, IRF6, PRKAR1A, SPINK1, or HBB.

In embodiments, the regulatory element comprises a secondary structure, such as a hairpin. In embodiments, the hairpin has a free energy of about –30, –40, –50, –60, –70, –80, –90, or –100 kcal/mol or stronger and is sufficient to reduce translation of the mRNA compared to an mRNA lacking the hairpin. In embodiments, the secondary structure is one found in TGF-beta1 mRNA, or a fragment or variant thereof, that binds YB-1.

In embodiments, the regulatory element comprises an RPB (RNA-binding protein) biding motif. In embodiments, the RNA binding protein comprises HuR, Musashi, an IRP (e.g., IRP1

or IRP2), SXL, or lin-14. In embodiments, the regulatory element comprises an IRE, SXL binding motif, p21 5' UTR GC-rich stem loop, or lin-4 motif. IRP1 and IRP2 bind to a stem-loop sequence called an iron-response element (IRE); binding creates a steric block to translation. The SXL protein binds a SXL binding motif, e.g., a poly-U stretches in an intron in the 5' UTR, causing intron retention. The SXL protein also binds a poly-U region in the 3' UTR, to block recruitment of the pre-initiation complex and repress translation. SXL also promotes translation of a uORF, repressing translation of the main coding region. The p21 5' UTR GC-rich stem loop is bound by CUGBP1 (a translational activator) or calreticulin (CRT, a translational repressor).

In some embodiments, the regulatory element comprises a binding site for a trans-acting RNA. In some embodiments, the trans-acting RNA is a miRNA. In embodiments, the untranslated region comprises an RNA-binding sequence, e.g., the lin-14 3' UTR which comprises conserved sequences that are bound by lin-4 RNA, thereby down-regulating translation of the lin-14 RNA (Wightman et al., Cell, Vol. 75, 855–862, December 3, 1993).

In embodiments, the regulatory element comprises a sequence that binds ribosomal RNA, e.g., that promotes shunting of the ribosome to bypass a segment of the 5' UTR and arrive at the start codon. In embodiments, the regulatory sequence that promotes shunting is a sequence found in cauliflower mosaic virus or adenovirus.

UTRs of red blood cell proteins

In some embodiments, the untranslated region is a UTR of an RNA that is expressed in a wild-type erythroid cell, e.g., in a mature red blood cell. In embodiments, the UTR is a UTR of a gene for a type I red blood cell transmembrane protein (e.g., glycophorin A), a type II red blood cell transmembrane protein (e.g., Kell or CD71), or a type III red blood cell transmembrane protein such as GLUT1. In embodiments, the UTR is a UTR of a red blood cell protein such as CD235a, c-Kit, GPA, IL3R, CD34, CD36, CD71, Band 3, hemoglobin, and Alpha 4 integrin. In embodiments, the UTR is a UTR of a gene for spectrin, ankyrin, 4.1R, 4.2, p55, tropomodulin, or 4.9.

In some embodiments, the untranslated region comprises a hemoglobin UTR, e.g., the 3' hemoglobin UTR of SEQ ID NO: 1:
GCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACT

ACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAA ACATTTATTTTCATTGC. In embodiments, the untranslated region comprises a stretch of at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, or 130 nucleotides of SEQ ID NO: 1. In embodiments, the untranslated region comprises a sequence with at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the sequence of SEQ ID NO: 1.

In some embodiments, the exogenous mRNA comprises a heterologous 3' UTR. In some embodiments, the exogenous mRNA comprises a heterologous 5' UTR. In some embodiments, the exogenous mRNA comprises a heterologous 3' UTR and a heterologous 5' UTR.

Regulatory RNAs

The invention includes, in some aspects, an erythroid cell comprising a regulatory RNA. In some embodiments, the cell further comprises an exogenous mRNA.

In related aspects, the invention includes a method of contacting an erythroid cell with a regulatory RNA. In embodiments, the method further comprises contacting the cell with an exogenous mRNA. In embodiments, the cell is contacted with the exogenous mRNA before, during, or after the contacting with the regulatory RNA.

In related aspects, the invention includes a composition (e.g., a purified or isolated composition) comprising: (i) a regulatory RNA (e.g., a miRNA or an anti-miR), and (ii) an exogenous mRNA described herein, e.g., an mRNA that is codon-optimized for expression in a human cell (e.g., in a human erythroid cell), an mRNA comprising a red blood cell transmembrane segment, or an mRNA comprising a heterologous UTR described herein (such as a hemoglobin UTR or a UTR from another red blood cell protein).

In embodiments, the regulatory RNA modulates a property (e.g., stability or translation) of the exogenous mRNA. In some embodiments, the regulatory RNA affects the erythroid cell, e.g., affects its proliferation or differentiation. In some embodiments, affecting proliferation comprises increasing the number of divisions a starting cell makes (e.g., in culture) and/or increasing the total number of cells produced from a starting cell or population. In some embodiments, regulating differentiation comprises promoting maturation and/or enucleation. In some embodiments, the regulatory RNA encodes EPO and, e.g., stimulates expansion of erythroid cells.

In embodiments, the regulatory RNA is a miRNA. In some embodiments, the miRNA is a human miRNA, e.g., an miRNA listed in Table 12 herein, e.g., one of the elements of Table 12 with a designation beginning with "MIR", or a sequence with no more than 1, 2, 3, 4, or 5 alterations (e.g., substitutions, insertions, or deletions) relative thereto.

In some embodiments, the regulatory RNA is an anti-miR. In some embodiments, an anti-miR inhibits a miRNA (such as an endogenous miRNA) by hybridizing with the miRNA and preventing the miRNA from binding its target mRNA. In some embodiments, the anti-miR binds and/or has complementarity to a human miRNA, e.g., an miRNA listed in Table 12 herein, e.g., one of the elements of Table 12 with a designation beginning with "MIR" , or a sequence with no more than 1, 2, 3, 4, or 5 alterations (e.g., substitutions, insertions, or deletions) relative thereto.

In some embodiments, the regulatory RNA is a siRNA, shRNA, or antisense molecule. In embodiments, the siRNA comprises a sense strand and an antisense strand which can hybridize to each other, wherein the antisense strand can further hybridize to a target mRNA; may have one or two blunt ends; may have one or two overhangs such as 3' dTdT overhangs; may comprise chemical modifications; may comprise a cap; and may comprise a conjugate. In embodiments, the shRNA comprises a hairpin structure with a sense region, an antisense region, and a loop region, wherein the sense region and antisense region can hybridize to each other, wherein the antisense region can further hybridize to a target mRNA; may have a blunt end; may have an overhang; may comprise chemical modifications; may comprise a cap; and may comprise a conjugate. In embodiments, the antisense molecule comprises a single strand that can hybridize to a target mRNA; may comprise chemical modifications; may comprise a cap; and may comprise a conjugate.

Lipid nanoparticle methods

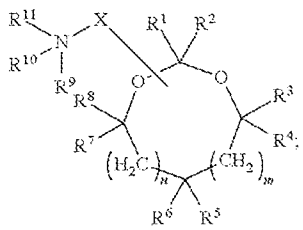
In some embodiments, an RNA (e.g., mRNA) described herein is introduced into an erythroid cell using lipid nanoparticle (LNPs), e.g., by transfection. Thus, in some aspects, the disclosure provides a method of introducing an mRNA encoding an exogenous protein into an erythroid cell, comprising contacting the erythroid cell with the mRNA and an LNP, e.g., an LNP described herein. The disclosure also provides reaction mixtures comprising an erythroid cell, an mRNA, and an LNP. In some embodiments, the mRNA is complexed with the LNP. In

embodiments, the population of cells contacted with the LNPs comprises at least 1 x 10^7, 2 x 10^7, 5 x 10^7, 1 x 10^8, 2 x 10^8, 5 x 10^8, 1 x 10^9, 2 x 10^9, or 5 x 10^9, 1 x 10^{10}, 2 x 10^{10}, or 5 x 10^{10} cells.

An exemplary LNP comprises a cationic trialkyl lipid, a non-cationic lipid (e.g., PEG-lipid conjugate and a phospholipid), and an mRNA molecule that is encapsulated within the lipid particle. In embodiments, the phospholipid comprises dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), or a mixture thereof. In embodiments, the PEG-lipid conjugate is selected from the group consisting of a PEG-diacylglycerol (PEG-DAG) conjugate, a PEG-dialkyloxypropyl (PEG-DAA) conjugate, a PEG-phospholipid conjugate, a PEG-ceramide (PEG-Cer) conjugate, and a mixture thereof. In embodiments, the PEG-DAA conjugate is selected from the group consisting of a PEG-didecyloxypropyl (C_{10}) conjugate, a PEG-dilauryloxypropyl (C_{12}) conjugate, a PEG-dimyristyloxypropyl (C_{14}) conjugate, a PEG-dipalmityloxypropyl (C_{16}) conjugate, a PEG-distearyloxypropyl (C_{18}) conjugate, and a mixture thereof. In embodiments, the LNP further comprises cholesterol. Additional LNPs are described, e.g., in US Pat. Pub. 20160256567, which is herein incorporated by reference in its entirety.

Another exemplary LNP can comprise a lipid having a structural Formula (I):

(I)



or salts thereof, wherein:

R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 are independently selected from the group consisting of hydrogen, optionally substituted C_7-C_{30} alkyl, optionally substituted C_7-C_{30} alkenyl and optionally substituted C_7-C_{30} alkynyl;

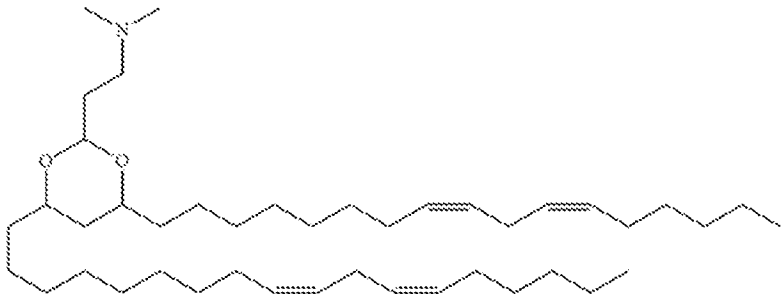
provided that (a) at least two of R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 are not hydrogen, and (b) two of the at least two of R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 that are not hydrogen are present in a 1, 3 arrangement, a 1, 4 arrangement or a 1, 5 arrangement with respect to each other;

X is selected from the group consisting of C_1-C_6 alkyl, C_2-C_6 alkenyl and C_2-C_6 alkynyl;

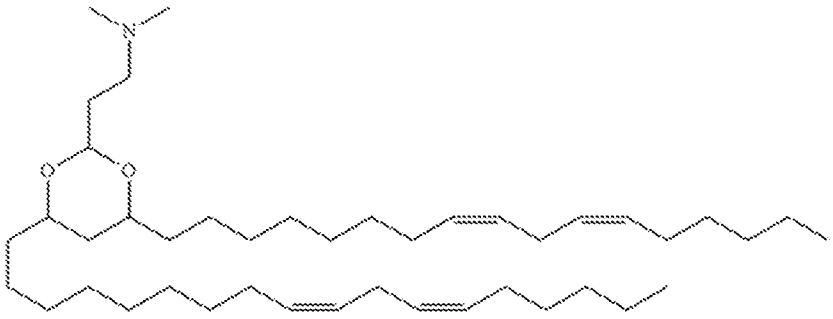
R^9, R^{10}, and R^{11} are independently selected from the group consisting of hydrogen, optionally substituted C_1-C_7 alkyl, optionally substituted C_2-C_7 alkenyl and optionally substituted C_2-C_7 alkynyl, provided that one of R^9, R^{10}, and R^{11} may be absent; and

n and m are each independently 0 or 1.

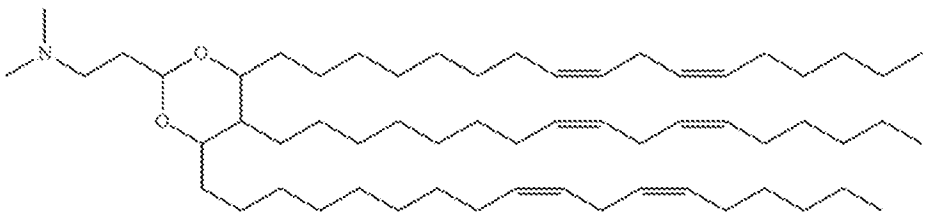
For instance, the lipid can comprise one of the following structures:



,



, or



In embodiments, the LNP further comprises a non-cationic lipid such as a phospholipid, cholesterol, or a mixture of a phospholipid and cholesterol. In embodiments, the phospholipid comprises dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), or a

mixture thereof. Additional LNPs are described, e.g., in US Pat. Pub. 20130064894, which is herein incorporated by reference in its entirety.

Another exemplary LNP comprises: (a) a nucleic acid, e.g., mRNA; (b) a cationic lipid comprising from 50 mol % to 65 mol % (e.g., 52 mol % to 62 mol %) of the total lipid present in the particle; (c) a non-cationic lipid comprising a mixture of a phospholipid and cholesterol or a derivative thereof, wherein the phospholipid comprises from 4 mol % to 10 mol % of the total lipid present in the particle and the cholesterol or derivative thereof comprises from 30 mol % to 40 mol % of the total lipid present in the particle; and (d) a conjugated lipid that inhibits aggregation of particles comprising from 0.5 mol % to 2 mol % of the total lipid present in the particle. In embodiments, the phospholipid comprises dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), or a mixture thereof. In embodiments, the conjugated lipid that inhibits aggregation of particles comprises a polyethyleneglycol (PEG)-lipid conjugate. In embodiments, the PEG-lipid conjugate comprises a PEG-diacylglycerol (PEG-DAG) conjugate, a PEG-dialkyloxypropyl (PEG-DAA) conjugate, or a mixture thereof. In embodiments, the PEG-DAA conjugate comprises a PEG-dimyristyloxypropyl (PEG-DMA) conjugate, a PEG-distearyloxypropyl (PEG-DSA) conjugate, or a mixture thereof. Additional LNPs are described, e.g., in US Pat. 8,058,069, which is herein incorporated by reference in its entirety.

Methods of manufacturing erythroid cells

Methods of differentiating erythroid precursor cells into mature erythroid cells are known. See, for example, Douay & Andreu. Transfus Med Rev. 2007 Apr;21(2):91-100; Giarratana et al. Nat Biotechnol. 2005 Jan;23(1):69-74; Olivier et al. Stem Cells Transl Med. 2012 Aug; 1(8): 604–614, and references cited therein.

Methods of manufacturing erythroid cells comprising (e.g., expressing) exogenous RNAs and/or proteins are described, e.g., in WO2015/073587 and WO2015/153102, each of which is incorporated by reference in its entirety.

In some embodiments, hematopoietic progenitor cells, e.g., CD34+ hematopoietic progenitor cells, are contacted with a nucleic acid or nucleic acids encoding one or more exogenous polypeptides, and the cells are allowed to expand and differentiate in culture.

In embodiments, the method comprises a step of electroporating the cells, e.g., as described herein.

In some embodiments, the erythroid cells are expanded at least 1000, 2000, 5000, 10,000, 20,000, 50,000, or 100,000 fold (and optionally up to 100,000, 200,000, or 500,000 fold). Number of cells is measured, in some embodiments, using an automated cell counter.

In some embodiments, the population of erythroid cells comprises at least 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 98% (and optionally up to about 80, 90, or 100%) enucleated cells. In some embodiments, the population of erythroid cells contains less than 1% live enucleated cells, e.g., contains no detectable live enucleated cells. Enucleation is measured, in some embodiments, by FACS using a nuclear stain. In some embodiments, at least 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, or 80% (and optionally up to about 70, 80, 90, or 100%) of erythroid cells in the population comprise an exogenous RNA and/or polypeptide. Expression of an exogenous polypeptide is measured, in some embodiments, by FACS using labeled antibodies against the polypeptide. In some embodiments, the population of enucleated cells comprises about $1x10^9 – 2x10^9$, $2x10^9 – 5x10^9$, $5x10^9 – 1x10^{10}$, $1x10^{10} – 2x10^{10}$, $2x10^{10} – 5x10^{10}$, $5x10^{10} – 1x10^{11}$, $1x10^{11} – 2x10^{11}$, $2x10^{11} – 5x10^{11}$, $5x10^{11} – 1x10^{12}$, $1x10^{12} – 2x10^{12}$, $2x10^{12} – 5x10^{12}$, or $5x10^{12} – 1x10^{13}$ cells.

Exemplary exogenous polypeptides and uses thereof

One or more of the exogenous proteins may have post-translational modifications characteristic of eukaryotic cells, e.g., mammalian cells, e.g., human cells. In some embodiments, one or more (e.g., 2, 3, 4, 5, or more) of the exogenous proteins are glycosylated, phosphorylated, or both. In vitro detection of glycoproteins is routinely accomplished on SDS-PAGE gels and Western Blots using a modification of Periodic acid-Schiff (PAS) methods. Cellular localization of glycoproteins may be accomplished utilizing lectin fluorescent conjugates known in the art. Phosphorylation may be assessed by Western blot using phospho-specific antibodies.

Post-translation modifications also include conjugation to a hydrophobic group (e.g., myristoylation, palmitoylation, isoprenylation, prenylation, or glypiation), conjugation to a cofactor (e.g., lipoylation, flavin moiety (e.g., FMN or FAD), heme C attachment, phosphopantetheinylation, or retinylidene Schiff base formation), diphthamide formation,

ethanolamine phosphoglycerol attachment, hypusine formation, acylation (e.g. O-acylation, N-acylation, or S-acylation), formylation, acetylation, alkylation (e.g., methylation or ethylation), amidation, butyrylation, gamma-carboxylation, malonylation, hydroxylation, iodination, nucleotide addition such as ADP-ribosylation, oxidation, phosphate ester (O-linked) or phosphoramidate (N-linked) formation, (e.g., phosphorylation or adenylylation), propionylation, pyroglutamate formation, S-glutathionylation, S-nitrosylation, succinylation, sulfation, ISGylation, SUMOylation, ubiquitination, Neddylation, or a chemical modification of an amino acid (e.g., citrullination, deamidation, eliminylation, or carbamylation), formation of a disulfide bridge, racemization (e.g., of proline, serine, alanine, or methionine). In embodiments, glycosylation includes the addition of a glycosyl group to arginine, asparagine, cysteine, hydroxylysine, serine, threonine, tyrosine, or tryptophan, resulting in a glycoprotein. In embodiments, the glycosylation comprises, e.g., O-linked glycosylation or N-linked glycosylation.

In some embodiments, one or more of the exogenous polypeptides is a fusion protein, e.g., is a fusion with an endogenous red blood cell protein or fragment thereof, e.g., a transmembrane protein, e.g., GPA or a transmembrane fragment thereof.

In some embodiments, the coding region for the exogenous polypeptide is codon-optimized for the cell in which it is expressed, e.g., a mammalian erythroid cell, e.g., a human erythroid cell.

In some embodiments, the erythroid cells comprise at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the exogenous protein per cell. In embodiments, the copy number of the exogenous protein can be determined, e.g., by quantitative Western blot or using standardized fluorescent microspheres (e.g., from Bangs Laboratories) in a flow cytometry assay. In some embodiments, e.g., wherein the exogenous protein is a fluorescent protein, the mean fluorescent intensity (MFI) can be used to estimate protein copy number, e.g., by determining the MFI of a sample, quantifying the copy number of the fluorescent protein in a similar sample (e.g., by quantitative Western blot), and calculating a conversion factor between MFI and protein copy number.

Physical characteristics of enucleated erythroid cells

In some embodiments, the erythroid cells described herein have one or more (e.g., 2, 3, 4, or more) physical characteristics described herein, e.g., osmotic fragility, cell size, hemoglobin concentration, or phosphatidylserine content. While not wishing to be bound by theory, in some embodiments an enucleated erythroid cell that expresses an exogenous protein has physical characteristics that resemble a wild-type, untreated erythroid cell (e.g., an erythroid cell not subjected to hypotonic dialysis). In contrast, a hypotonically loaded RBC sometimes displays altered physical characteristics such as increased osmotic fragility, altered cell size, reduced hemoglobin concentration, or increased phosphatidylserine levels on the outer leaflet of the cell membrane.

Osmotic fragility

In some embodiments, the enucleated erythroid cell exhibits substantially the same osmotic membrane fragility as an isolated, uncultured erythroid cell that does not comprise an exogenous polypeptide. In some embodiments, the population of enucleated erythroid cells have an osmotic fragility of less than 50% cell lysis at 0.3%, 0.35%, 0.4%, 0.45%, or 0.5% NaCl. In some embodiments, the population of enucleated erythroid cells has an osmotic fragility of less than 50% cell lysis in a solution consisting of 0.3%, 0.35%, 0.4%, 0.45%, or 0.5% NaCl in water. Osmotic fragility is determined, in some embodiments, using the method of Example 59 of WO2015/073587.

Cell size

In some embodiments, the enucleated erythroid cell has approximately the diameter or volume as a wild-type, untreated reticulocyte. In some embodiments, the enucleated erythroid cell has a volume of about 150 fL, e.g., about 140-160, 130-170, or 120-180 fL. In some embodiments, the population has a mean cell volume of about 150 fL, about 140-160, 130-170, 120-180, 110-190, or 100-200 fL. In some embodiments, at least about 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the cells in the population have a volume of between about 140-160, 130-170, 120-180, 110-190, or 100-200 fL. In some embodiment the volume of the mean corpuscular volume of the erythroid cells is greater than 10 fL, 20 fL, 30 fL, 40 fL, 50 fL, 60 fL, 70 fL, 80 fL, 90 fL, 100 fL, 110 fL, 120 fL, 130 fL, 140 fL, 150 fL, or greater than 150 fL. In

one embodiment the mean corpuscular volume of the erythroid cells is less than 30 fL, 40 fL, 50 fL, 60 fL, 70 fL, 80 fL, 90 fL, 100 fL, 110 fL, 120 fL, 130 fL, 140 fL, 150 fL, 160 fL, 170 fL, 180 fL, 190 fL, 200 fL, or less than 200 fL. In one embodiment the mean corpuscular volume of the erythroid cells is between 80 – 100, 100-200, 200-300, 300-400, or 400-500 femtoliters (fL). In some embodiments, a population of erythroid cells has a mean corpuscular volume set out in this paragraph and the standard deviation of the population is less than 50, 40, 30, 20, 10, 5, or 2 fL. Volume is measured, in some embodiments, using a hematological analysis instrument, e.g., a Coulter counter.

Hemoglobin concentration

In some embodiments, the enucleated erythroid cell has a hemoglobin content similar to a wild-type, untreated erythroid cell, e.g., a mature RBC. In some embodiments, the erythroid cell comprises greater than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or greater than 10% fetal hemoglobin. In some embodiments, the erythroid cell comprises at least about 20, 22, 24, 26, 28, or 30 pg, and optionally up to about 30 pg, of total hemoglobin. Hemoglobin levels are determined, in some embodiments, using the Drabkin's reagent method of Example 33 of WO2015/073587.

Phosphatidylserine content

In some embodiments, the enucleated erythroid cell has approximately the same phosphatidylserine content on the outer leaflet of its cell membrane as a wild-type, untreated RBC. Phosphatidylserine is predominantly on the inner leaflet of the cell membrane of wild-type, untreated RBCs, and hypotonic loading can cause the phosphatidylserine to distribute to the outer leaflet where it can trigger an immune response. In some embodiments, the population of RBC comprises less than about 30, 25, 20, 15, 10, 9, 8, 6, 5, 4, 3, 2, or 1% of cells that are positive for Annexin V staining. In some embodiments, at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the enucleated cells in the population have the same as level of phosphatidylserine exposure as an otherwise similar cultured erythroid cell that does not express an exogenous protein. Phosphatidylserine exposure is assessed, in some embodiments, by staining for Annexin-V-FITC, which binds

preferentially to PS, and measuring FITC fluorescence by flow cytometry, e.g., using the method of Example 54 of WO2015/073587.

Other characteristics

In some embodiments, the population of erythroid cells comprises at least about 50%, 60%, 70%, 80%, 90%, or 95% (and optionally up to 90 or 100%) of cells that are positive for GPA. The presence of GPA is detected, in some embodiments, using FACS.

In some embodiments, the erythroid cells have a half-life of at least 30, 45, or 90 days in a subject.

Phases of erythroid cell differentiation and maturation

In embodiments, enucleated erythroid cells are produced by exposing CD34+ stem cells to three conditions: first expansion, then differentiation, and finally maturation conditions. Exemplary expansion, differentiation, and maturation conditions are described, e.g., as steps 1, 2, and 3 respectively in Example 3, paragraph [1221] of WO2015/073587, which is herein incorporated by reference in its entirety. In embodiments, expansion phase comprises culturing the cells in an expansion medium (e.g., medium of step 1 above), differentiation phase comprises culturing the cells in a differentiation medium (e.g., medium of step 2 above), and maturation phase comprises culturing the cells in a maturation medium (e.g., medium of step 3 above).

In embodiments, maturation phase begins when about 84% of the cells in the population are positive for GPA, e.g., as measured by a flow cytometry assay. In embodiments, at the beginning of maturation phase, a population of cells is about 54% band3-positive, e.g., as measured by a flow cytometry assay. In embodiments, at the beginning of maturation phase, a population of cells is about 98% alpha4 integrin-positive, e.g., as measured by a flow cytometry assay. In an embodiment, maturation phase begins when about 53% of cells in the erythroid cell population are positive for both band3 and alpha4 integrin. In embodiments, maturation phase begins when the cell population is predominantly pre-erythroblasts and basophilic erythroblasts.

In embodiments, about 99% of cells in an erythroid cell population described herein are positive for GPA. In an embodiment, about 98% of cells in the erythroid cell population are positive for band3. In an embodiment, about 91% of cells in the erythroid cell population are positive for alpha4 integrin. In an embodiment, about 90% of cells in the erythroid cell

population are positive for both band3 and alpha4 integrin. In embodiments, the cell population is predominantly polychromatic erythroblasts and orthochromatic erythroblasts. In embodiments, the cell population is about 3% enucleated. In embodiments, the cell population is at about 6% of maximal enucleation, wherein maximal enucleation is the percentage enucleation the cell population reaches at the end of culturing. In embodiments, the cell population has an AHA intensity/incorporation value of about 2,410,000 in a BONCAT assay, e.g., as described in Example 10. In embodiments, this stage is reached when the cells have been exposed to maturation conditions for 3 days (day M3).

In embodiments, about 99.5% of cells in an erythroid cell population described herein are positive for GPA. In an embodiment, about 100% of cells in the erythroid cell population are positive for band3. In an embodiment, about 84.2% of cells in the erythroid cell population are positive for alpha4 integrin. In an embodiment, about 84.2% of cells in the erythroid cell population are positive for both band3 and alpha4 integrin. In embodiments, the cell population is predominantly orthochromatic erythroblasts and reticulocytes. In embodiments, the cell population is about 11% enucleated. In embodiments, the cell population is at about 22% of maximal enucleation. In embodiments, the cell population has an AHA intensity/incorporation value of about 1,870,000 in a BONCAT assay. In embodiments, this stage is reached when the cells have been exposed to maturation conditions for 5 days (day M5).

In embodiments, the cell population is about 34% enucleated. In embodiments, the cell population is at about 68% of maximal enucleation. In embodiments, the cell population has an AHA intensity/incorporation value of about 615,000 in a BONCAT assay. In embodiments, this stage is reached when the cells have been exposed to maturation conditions for 7 days (day M7).

In embodiments, the cell population is about 43% enucleated. In embodiments, the cell population is at about 86% of maximal enucleation. In embodiments, the cell population has an AHA intensity/incorporation value of about 189,000 in a BONCAT assay. In embodiments, this stage is reached when the cells have been exposed to maturation conditions for 9 days (day M9).

In embodiments, an erythroid cell is selected from a pro-erythroblast, early basophilic erythroblast, late basophilic erythroblast, polychromatic erythroblast, orthochromatic erythroblast, reticulocyte, or erythrocyte.

Methods of treatment with compositions herein, e.g., erythroid cells

Methods of administering erythroid cells comprising (e.g., expressing) an exogenous RNA and/or protein are described, e.g., in WO2015/073587 and WO2015/153102, each of which is incorporated by reference in its entirety.

In embodiments, the erythroid cells described herein are administered to a subject, e.g., a mammal, e.g., a human. Exemplary mammals that can be treated include without limitation, humans, domestic animals (e.g., dogs, cats and the like), farm animals (e.g., cows, sheep, pigs, horses and the like) and laboratory animals (e.g., monkey, rats, mice, rabbits, guinea pigs and the like). The methods described herein are applicable to both human therapy and veterinary applications.

In some embodiments, the erythroid cells are administered to a patient every 1, 2, 3, 4, 5, or 6 months.

In some embodiments, a dose of erythroid cells comprises about $1x10^9 – 2x10^9$, $2x10^9 – 5x10^9$, $5x10^9 – 1x10^{10}$, $1x10^{10} – 2x10^{10}$, $2x10^{10} – 5x10^{10}$, $5x10^{10} – 1x10^{11}$, $1x10^{11} – 2x10^{11}$, $2x10^{11} – 5x10^{11}$, $5x10^{11} – 1x10^{12}$, $1x10^{12} – 2x10^{12}$, $2x10^{12} – 5x10^{12}$, or $5x10^{12} – 1x10^{13}$ cells.

In some aspects, the present disclosure provides a method of treating a disease or condition described herein, comprising administering to a subject in need thereof a composition described herein, e.g., an enucleated red blood cell described herein. In some embodiments, the disease or condition is cancer, an infection (e.g., a viral or bacterial infection), an inflammatory disease, an autoimmune disease, or a metabolic deficiency. In some aspects, the disclosure provides a use of an erythroid cell described herein for treating a disease or condition described herein. In some aspects, the disclosure provides a use of an erythroid cell described herein for manufacture of a medicament for treating a disease or condition described herein.

Types of cancer include acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), anal cancer, bile duct cancer, bladder cancer, bone cancer, bowel cancer, brain tumors, breast cancer, cancer of unknown primary, cancer spread to bone, cancer spread to brain, cancer spread to liver, cancer spread to lung, carcinoid, cervical cancer, choriocarcinoma, chronic lymphocytic leukaemia (CLL), chronic myeloid leukaemia (CML), colon cancer, colorectal cancer, endometrial cancer, eye cancer, gallbladder cancer, gastric cancer, gestational trophoblastic tumors (GTT), hairy cell leukaemia, head and neck cancer, Hodgkin lymphoma,

kidney cancer, laryngeal cancer, leukaemia, liver cancer, lung cancer, lymphoma, melanoma skin cancer, mesothelioma, men's cancer, molar pregnancy, mouth and oropharyngeal cancer, myeloma, nasal and sinus cancers, nasopharyngeal cancer, non-Hodgkin lymphoma (NHL), esophageal cancer, ovarian cancer, pancreatic cancer, penile cancer, prostate cancer, rare cancers, rectal cancer, salivary gland cancer, secondary cancers, skin cancer (non-melanoma), soft tissue sarcoma, stomach cancer, testicular cancer, thyroid cancer, unknown primary cancer, uterine cancer, vaginal cancer, and vulval cancer.

Viral infections include adenovirus, coxsackievirus, hepatitis A virus, poliovirus, Epstein-Barr virus, herpes simplex type 1, herpes simplex type 2, human cytomegalovirus, human herpesvirus type 8, varicella-zoster virus, hepatitis B virus, hepatitis C viruses, human immunodeficiency virus (HIV), influenza virus, measles virus, mumps virus, parainfluenza virus, respiratory syncytial virus, papillomavirus, rabies virus, and Rubella virus. Other viral targets include Paramyxoviridae (e.g., pneumovirus, morbillivirus, metapneumovirus, respirovirus or rubulavirus), Adenoviridae (e.g., adenovirus), Arenaviridae (e.g., arenavirus such as lymphocytic choriomeningitis virus), Arteriviridae (e.g., porcine respiratory and reproductive syndrome virus or equine arteritis virus), Bunyaviridae (e.g., phlebovirus or hantavirus), Caliciviridae (e.g., Norwalk virus), Coronaviridae (e.g., coronavirus or torovirus), Filoviridae (e.g., Ebola-like viruses), Flaviviridae (e.g., hepacivirus or flavivirus), Herpesviridae (e.g., simplexvirus, varicellovirus, cytomegalovirus, roseolovirus, or lymphocryptovirus), Orthomyxoviridae (e.g., influenza virus or thogotovirus), Parvoviridae (e.g., parvovirus), Picomaviridae (e.g., enterovirus or hepatovirus), Poxviridae (e.g., orthopoxvirus, avipoxvirus, or leporipoxvirus), Retroviridae (e.g., lentivirus or spumavirus), Reoviridae (e.g., rotavirus), Rhabdoviridae (e.g., lyssavirus, novirhabdovirus, or vesiculovirus), and Togaviridae (e.g., alphavirus or rubivirus). Specific examples of these viruses include human respiratory coronavirus, influenza viruses A-C, hepatitis viruses A to G, and herpes simplex viruses 1-9.

Bacterial infections include, but are not limited to, Mycobacteria, Rickettsia, Mycoplasma, Neisseria meningitides, Neisseria gonorrheoeae, Legionella, Vibrio cholerae, Streptococci, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Corynobacteria diphtheriae, Clostridium spp., enterotoxigenic Eschericia coli, Bacillus anthracis, Rickettsia, Bartonella henselae, Bartonella quintana, Coxiella burnetii, chlamydia, Mycobacterium leprae, Salmonella; shigella; Yersinia enterocolitica; Yersinia pseudotuberculosis; Legionella

pneumophila; Mycobacterium tuberculosis; Listeria monocytogenes; Mycoplasma spp.; Pseudomonas fluorescens; Vibrio cholerae; Haemophilus influenzae; Bacillus anthracis; Treponema pallidum; Leptospira; Borrelia; Corynebacterium diphtheriae; Francisella; Brucella melitensis; Campylobacter jejuni; Enterobacter; Proteus mirabilis; Proteus; and Klebsiella pneumoniae.

Inflammatory disease include bacterial sepsis, rheumatoid arthritis, age related macular degeneration (AMD), systemic lupus erythematosus (an inflammatory disorder of connective tissue), glomerulonephritis (inflammation of the capillaries of the kidney), Crohn's disease, ulcerative colitis, celiac disease, or other idiopathic inflammatory bowel diseases, and allergic asthma.

Autoimmune diseases include systemic lupus erythematosus, glomerulonephritis, rheumatoid arthritis, multiple sclerosis, and type 1 diabetes.

Metabolic deficiencies include Phenylketonuria (PKU), Adenosine Deaminase Deficiency-Severe Combined Immunodeficiency (ADA-SCID), Mitochondrial Neurogastrointestinal Encephalopathy (MNGIE), Primary Hyperoxaluria, Alkaptonuria, and Thrombotic Thrombocytopenic Purpura (TTP).

Exemplary additional features and embodiments are provided below:

1. A method of making an erythroid cell comprising a nucleic acid, e.g., an mRNA, encoding an exogenous protein, comprising:

a) providing an erythroid cell in maturation phase, and

b) contacting the erythroid cell with a nucleic acid, e.g., an mRNA, encoding the exogenous protein, under conditions that allow uptake of the nucleic acid, e.g., an mRNA, by the erythroid cell,

thereby making an erythroid cell comprising a nucleic acid, e.g., an mRNA, encoding an exogenous protein.

2. The method of embodiment 1, wherein the erythroid cell takes up the nucleic acid, e.g., an mRNA, encoding the exogenous protein.

3. The method of embodiment 1, comprising providing a population of erythroid cells in maturation phase and contacting a plurality of cells of the population of erythroid cells with the nucleic acid, e.g., an mRNA, encoding the exogenous protein.

4. The method of embodiment 3, wherein the plurality of cells of the population of erythroid cells each takes up the nucleic acid, e.g., an mRNA, encoding the exogenous protein.

5. The method of any of embodiments 1-4, wherein after uptake of the nucleic acid, e.g., an mRNA, encoding the exogenous protein, the cell or the plurality of cells express the exogenous protein.

6. The method of embodiment 5, wherein the cell or the plurality of cells comprise the exogenous protein.

7. The method of any of embodiments 3-6, wherein the population of erythroid cells in maturation phase is a population of cells expanded in a maturation medium for 3-7 days, e.g., 4-5 or 4-6 days.

8. A method of manufacturing a population of reticulocytes that express an exogenous protein, the method comprising

(a) providing a population of erythroid precursor cells (e.g., CD34+ cells);
(b) culturing the population of erythroid precursor cells under differentiating conditions to provide a population of differentiating erythroid cells;
(c) contacting a plurality of cells of the population of differentiating erythroid cells with a nucleic acid, e.g., an mRNA, encoding the exogenous protein, under conditions that allow uptake of the nucleic acid, e.g., an mRNA, by the plurality of cells of the population of differentiating erythroid cells; and
(d) further culturing the plurality of cells of the population of differentiating erythroid cells to provide a population of reticulocytes,

thereby manufacturing a population of reticulocytes that express the exogenous protein.

9. The method of embodiment 8, wherein the further culturing comprises fewer than 3, 2, or 1 population doubling.

10. The method of any of embodiments 3-9, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more) of the following properties:

i.a) 2-40%, 3-33%, 5-30%, 10-25%, or 15-20% of the cells in the population are enucleated;

i.b) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 2%, 3%, 4%, or 5% of the cells in the population are enucleated;

i.c) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 6%, 10%, 15%, 20%, or 25% of the cells in the population are enucleated;

i.d) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 30%, 35%, 40%, 45%, or 50% of the cells in the population are enucleated;

i.e) no more than 1%, 2%, 3%, 5%, 10%, 15%, or 20% of the cells in the population are enucleated;

i.f) no more than 25%, 30%, 35%, 40%, 45%, or 50% of the cells in the population are enucleated;

i.g) the population of cells has reached 6-70%, 10-60%, 20-50%, or 30-40% of maximal enucleation;

i.h) the population of cells has reached no more than 1%, 2%, 3%, 5%, 10%, 15%, or 20% of maximal enucleation;

i.i) the population of cells has reached no more than 25%, 30%, 35%, 40%, 45%, 50%, or 60% of maximal enucleation;

ii.a) the population of cells is fewer than 3, 2, or 1 population doubling from a plateau in cell division;

ii.b) the population of cells is capable of fewer than 3, 2, or 1 population doubling;

ii.c) the population will increase by no more than 1.5, 2, or 3 fold before the population reaches an enucleation level of at least 70% of cells in the population;

iii.a) at least 80%, 85%, 90%, 95%, or 99% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.b) at least 50%, 60%, 70%, 75%, or 79% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.c) 30-90%, 40-90%, 50-90%, 60-90%, or 70-90% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.d) at least 80%, 85%, 90%, 95%, or 99% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast);

iii.e) at least 50%, 60%, 70%, 75%, or 79% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast);

iii.f) 30-90%, 40-90%, 50-90%, 60-90%, or 70-90% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast);

iv.a) the population of cells has at least 60%, 70%, 80%, or 90% of maximal translational activity;

iv.b) the population of cells has at least 20%, 30%, 40%, or 50% of maximal translational activity;

iv.c) the population of cells has a translational activity of at least 600,000, 800,000, 1,000, 000, 1,200,000, 1,400,000, 1,600,000, 1,800,000, 2,000,000, 2,200,000, or 2,400,000, as measured by a BONCAT assay, e.g., by the translation assay of Example 10; or

iv. d) the population of cells has a translational activity of 600,000-2,400,000, 800,000-2,200,000, 1,000, 000-2,000,000, 1,200,000-1,800,000, or 1,400,000-1,600,000, as measured by a BONCAT assay, e.g., by the translation assay of Example 10.

11. The method of embodiment 10, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i and a property from ii.

12. The method of embodiment 10, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i and a property from iii.

13. The method of embodiment 10, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i and a property from iv.

14. The method of embodiment 10, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from ii and a property from iii.

15. The method of embodiment 10, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from ii and a property from iv.

16. The method of embodiment 10, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from iii and a property from iv.

17. The method of embodiment 10, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i, a property from ii, and a property from iii.

18. The method of embodiment 10, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i, a property from ii, and a property from iv.

19. The method of embodiment 10, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i, a property from iii, and a property from iv.

20. The method of embodiment 10, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from ii, a property from iii, and a property from iv.

21. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.a and ii.a.

22. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.b and ii.a.

23. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.c and ii.a.

24. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.d and ii.a.

25. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.e and ii.a.

26. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.f and ii.a.

27. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.g and ii.a.

28. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.h and ii.a.

29. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.i and ii.a.

30. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.a and ii.b.

31. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.b and ii.b.

32. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.c and ii.b.

33. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.d and ii.b.

34. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.e and ii.b.

35. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.f and ii.b.

36. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.g and ii.b.

37. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.h and ii.b.

38. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.i and ii.b.

39. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.a and ii.c.

40. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.b and ii.c.

41. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.c and ii.c.

42. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.d and ii.c.

43. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.e and ii.c.

44. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.f and ii.c.

45. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.g and ii.c.

46. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.h and ii.c.

47. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.i and ii.c.

48. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.a and iii.a.

49. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.b and iii.a.

50. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.c and iii.a.

51. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.d and iii.a.

52. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.e and iii.a.

53. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.f and iii.a.

54. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.g and iii.a.

55. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.h and iii.a.

56. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.i and iii.a.

57. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.a and iii.b.

58. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.b and iii.b.

59. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.c and iii.b.

60. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.d and iii.b.

61. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.e and iii.b.

62. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.f and iii.b.

63. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.g and iii.b.

64. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.h and iii.b.

65. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.i and iii.b.

66. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.a and iii.c.

67. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.b and iii.c.

68. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.c and iii.c.

69. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.d and iii.c.

70. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.e and iii.c.

71. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.f and iii.c.

72. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.g and iii.c.

73. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.h and iii.c.

74. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.i and iii.c.

75. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.a and iii.d.

76. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.b and iii.d.

77. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.c and iii.d.

78. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.d and iii.d.

79. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.e and iii.d.

80. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.f and iii.d.

81. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.g and iii.d.

82. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.h and iii.d.

83. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.i and iii.d.

84. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.a and iii.e.

85. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.b and iii.e.

86. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.c and iii.e.

87. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.d and iii.e.

88. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.e and iii.e.

89. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.f and iii.e.

90. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.g and iii.e.

91. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.h and iii.e.

92. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.i and iii.e.

93. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.a and iii.f.

94. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.b and iii.f.

95. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.c and iii.f.

96. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.d and iii.f.

97. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.e and iii.f.

98. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.f and iii.f.

99. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.g and iii.f.

100. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.h and iii.f.

101. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.i and iii.f.

102. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.a and iv.a.

103. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.b and iv.a.

104. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.c and iv.a.

105. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.d and iv.a.

106. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.e and iv.a.

107. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.f and iv.a.

108. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.g and iv.a.

109. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.h and iv.a.

110. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.i and iv.a.

111. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.a and iv.b.

112. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.b and iv.b.

113. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.c and iv.b.

114. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.d and iv.b.

115. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.e and iv.b.

116. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.f and iv.b.

117. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.g and iv.b.

118. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.h and iv.b.

119. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.i and iv.b.

120. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.a and iv.c.

121. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.b and iv.c.

122. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.c and iv.c.

123. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.d and iv.c.

124. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.e and iv.c.

125. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.f and iv.c.

126. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.g and iv.c.

127. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.h and iv.c.

128. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.i and iv.c.

129. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.a and iv.d.

130. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.b and iv.d.

131. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.c and iv.d.

132. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.d and iv.d.

133. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.e and iv.d.

134. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.f and iv.d.

135. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.g and iv.d.

136. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.h and iv.d.

137. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: i.i and iv.d.

138. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.a and ii.a.

139. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.b and ii.a.

140. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.c and ii.a.

141. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.d and ii.a.

142. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.e and ii.a.

143. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.f and ii.a.

144. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.a and ii.b.

145. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.b and ii.b.

146. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.c and ii.b.

147. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.d and ii.b.

148. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.e and ii.b.

149. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.f and ii.b.

150. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.a and ii.c.

151. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.b and ii.c.

152. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.c and ii.c.

153. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.d and ii.c.

154. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.e and ii.c.

155. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.f and ii.c.

156. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.a and iv.a.

157. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.b and iv.a.

158. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.c and iv.a.

159. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.d and iv.a.

160. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.e and iv.a.

161. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.f and iv.a.

162. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.a and iv.b.

163. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.b and iv.b.

164. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.c and iv.b.

165. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.d and iv.b.

166. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.e and iv.b.

167. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.f and iv.b.

168. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.a and iv.c.

169. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.b and iv.c.

170. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.c and iv.c.

171. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.d and iv.c.

172. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.e and iv.c.

173. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.f and iv.c.

174. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.a and iv.d.

175. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.b and iv.d.

176. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.c and iv.d.

177. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.d and iv.d.

178. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.e and iv.d.

179. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: iii.f and iv.d.

180. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: ii.a and iv.a.

181. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: ii.b and iv.a.

182. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: ii.c and iv.a.

183. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: ii.a and iv.b.

184. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: ii.b and iv.b.

185. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: ii.c and iv.b.

186. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: ii.a and iv.c.

187. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: ii.b and iv.c.

188. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: ii.c and iv.c.

189. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: ii.a and iv.d.

190. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: ii.b and iv.d.

191. The method of any of embodiments 10-20, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following properties: ii.c and iv.d.

192. The method of any of embodiments 3-191, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following property: 84-99%, 85-95%, or about 90% of the cells in the population are GPA-positive, e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10.

193. The method of any of embodiments 3-191, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following property: at least 84%, 85%, 90%, 95%, or 99% of the cells in the population are GPA-positive, e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10.

194. The method of any of embodiments 3-191, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following property: 54-99%, 55-98%, 60-95%, 65-90%, 70-85%, or 75-80% of the cells in the population are band3-positive, e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10.

195. The method of any of embodiments 3-191, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following property: at least 54%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% of the cells in the population are band3-positive, e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10.

196. The method of any of embodiments 3-191, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following property: 96-100%, 97-99%, or about 98% of the cells in the population are alpha4 integrin-positive, e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10.

197. The method of any of embodiments 3-191, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following property: at least 95%, 96%, 97%, 98%, or 99% of the cells in the population are alpha4 integrin-positive, e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10.

198. The method of any of embodiments 3-197, wherein prior to or after contacting the plurality of cells with the nucleic acid, e.g., an mRNA, encoding the exogenous protein, the plurality of cells are separated from the population of erythroid cells or the population of differentiating erythroid cells, e.g., the plurality of cells are separated from the population based on enucleation status (e.g., the plurality of cells are nucleated cells and the rest of the population are enucleated cells).

199. The method of any of embodiments 3-197, comprising prior to or after contacting the plurality of cells with the nucleic acid, e.g., an mRNA, encoding the exogenous protein, synchronizing the population of erythroid cells or the population of differentiating erythroid cells, e.g., by arresting the growth, development, hemoglobin synthesis, or the process of enucleation of the population, e.g., by incubating the population with an inhibitor of enucleation (e.g., an inhibitor of histone deacetylase (HDAC), an inhibitor of mitogen-activated protein kinase (MAPK), an inhibitor of cyclin-dependent kinase (CDK), or a proteasome inhibitor).

200. The method of embodiment 199, wherein arresting occurs prior to enucleation of more than 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10% of the cells in the population.

201. A method of manufacturing a population of reticulocytes that express an exogenous protein, the method comprising:

(e) providing a population of erythroid precursor cells (e.g., CD34+ cells);

(f) culturing the population of erythroid precursor cells under differentiating conditions to provide a population of differentiating erythroid cells;

(g) contacting the differentiating erythroid cells with an mRNA encoding the exogenous protein, under conditions that allow uptake of the mRNA by the differentiating erythroid cells, wherein the contacting is performed when the population of differentiating erythroid cells is between 0.1 and 25% enucleated (e.g., between 0.1 and 20% enucleated, between 0.1 and 15% enucleated, between 0.1 and 12% enucleated, or between 0.1 and 10% enucleated); and

(h) further culturing the differentiating erythroid cells to provide a population of reticulocytes,

thereby manufacturing a population of reticulocytes that express the exogenous protein.

202. The method of embodiment 201, wherein the further culturing comprises fewer than 3, 2, or 1 population doubling.

203. The method of embodiment 201 or 202, wherein the contacting is performed when at least 50% (at least 60%, 70%, 75%, 80%, 90%, or 95%) of the differentiating erythroid cells exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast).

204. A method of manufacturing a population of reticulocytes that express an exogenous protein, comprising (a) providing a population of erythroid precursor cells, (b) culturing the population of erythroid precursor cells under differentiating conditions to provide a population of differentiating erythroid cells, (c) contacting the differentiating erythroid cells with an mRNA encoding the exogenous protein, wherein the improvement comprises: the contacting is performed when the population of differentiating erythroid cells is between 0.1 and 25% enucleated (e.g., between 0.1 and 20% enucleated, between 0.1 and 15% enucleated, between 0.1 and 12% enucleated, or between 0.1 and 10% enucleated).

205. The method of embodiment 204, wherein the contacting is performed when the population of differentiating erythroid cells has fewer than 3, 2, or 1 population doubling before a plateau in cell division.

206. The method of embodiment 204 or 205, wherein the contacting is performed when at least 50% (at least 60%, 70%, 75%, 80%, 90%, or 95%) of the differentiating erythroid cells exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast).

207. An erythroid cell, e.g., an enucleated erythroid cell, comprising:

an exogenous mRNA comprising a coding region operatively linked to a heterologous untranslated region (UTR), wherein the heterologous UTR comprises a regulatory element.

208. An erythroid cell, e.g., an enucleated erythroid cell, comprising an exogenous mRNA that comprises one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof.

209. A method of producing an erythroid cell, e.g., enucleated erythroid cell, comprising:

a) contacting an erythroid cell, e.g., a nucleated erythroid cell, with an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element, (e.g., isolated RNA or in vitro transcribed RNA), and

b) maintaining the contacted erythroid cell under conditions suitable for uptake of the exogenous mRNA,

thereby producing the erythroid cell, e.g., an enucleated erythroid cell.

210. A method of producing an erythroid cell, e.g., enucleated erythroid cell, comprising:

a) contacting an erythroid cell, e.g., a nucleated erythroid cell, with an exogenous mRNA comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof; and

b) maintaining the contacted erythroid cell under conditions suitable for uptake of the exogenous mRNA,

thereby producing the erythroid cell, e.g., an enucleated erythroid cell.

211. A method of producing an exogenous protein in an enucleated erythroid cell:

a) providing an erythroid cell, e.g., a nucleated erythroid cell, comprising an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element, (e.g., isolated RNA or in vitro transcribed RNA), and

b) culturing the erythroid cell under conditions suitable for production of the exogenous protein,

thereby producing the exogenous protein.

212. A method of producing an exogenous protein in an enucleated erythroid cell:

a) providing an erythroid cell, e.g., a nucleated erythroid cell, comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof, and

b) culturing the erythroid cell under conditions suitable for production of the exogenous protein,

thereby producing the exogenous protein.

213. A method of providing a subject with an exogenous protein, providing a subject with an enucleated erythroid cell which can produce an exogenous protein, or treating a subject, comprising administering to the subject:

an erythroid cell, e.g., a nucleated erythroid cell, comprising an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element, (e.g., isolated RNA or in vitro transcribed RNA),

thereby providing a subject with an exogenous protein, providing a subject with an enucleated erythroid cell which can produce an exogenous protein, or treating a subject.

214. A method of providing a subject with an exogenous protein, providing a subject with an enucleated erythroid cell which can produce an exogenous protein, or treating a subject, comprising administering to the subject:

an erythroid cell, e.g., a nucleated erythroid cell, comprising an exogenous mRNA comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof,

thereby providing a subject with an exogenous protein, providing a subject with an enucleated erythroid cell which can produce an exogenous protein, or treating a subject.

215. A method of evaluating an erythroid cell, e.g., enucleated erythroid cell (or a batch of such cells) comprising:

a) providing an erythroid cell, e.g., a nucleated erythroid cell, comprising an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element (or a batch of such cells), and

b) evaluating the erythroid cell, e.g., the nucleated erythroid cell (or batch of such cells) for a preselected parameter,

thereby evaluating the erythroid cell, e.g., enucleated erythroid cell (or a batch of such cells).

216. A method of evaluating an erythroid cell, e.g., enucleated erythroid cell (or a batch of such cells) comprising:

a) providing an erythroid cell, e.g., a nucleated erythroid cell, comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof, and

b) evaluating the erythroid cell, e.g., the nucleated erythroid cell (or batch of such cells) for a preselected parameter,

thereby evaluating the erythroid cell, e.g., enucleated erythroid cell (or a batch of such cells).

217. The method of embodiment 207, wherein at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells in the population comprise the exogenous protein, e.g., 5 days after contacting with the mRNA.

218. The method of embodiment 207, wherein the cells in the population comprise at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the exogenous protein, e.g., 5 days after the contacting with the mRNA.

219. The method of embodiment 207, wherein the cells comprise at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the exogenous protein for at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days after contacting with the mRNA.

220. A method of making an erythroid cell comprising an mRNA that encodes an exogenous protein, comprising:

providing a reaction mixture comprising an erythroid cell and an mRNA encoding the exogenous protein, under conditions which inhibit degradation of mRNA, e.g., by inclusion in the reaction mixture a ribonuclease inhibitor, and

maintaining the reaction mixture under conditions that allow uptake of the mRNA by the erythroid cell,

thereby making an erythroid cell comprising an mRNA encoding an exogenous protein.

221. The method of embodiment 220, comprising providing a population of erythroid cells and contacting the population with the mRNA encoding the exogenous protein.

222. The method of embodiment 220 or 221, wherein a plurality of erythroid cells of the population each takes up an mRNA encoding the exogenous protein.

223. The method of any of embodiments 220-222, wherein the cell or plurality of cells express the exogenous protein.

224. The method of any of embodiments 220-223, wherein the cell or plurality of cells comprise the exogenous protein.

225. The method of any of embodiments 220-224, which further comprises electroporating the cell or population of cells.

226. The method of any of embodiments 220-225, which further comprises contacting a population of erythroid cells with a ribonuclease inhibitor.

227. The method of any of embodiments 220-226, which comprises contacting the population of cells with the ribonuclease inhibitor before, during, or after contacting the cells with the mRNA.

228. The method of any of embodiments 220-227, which comprises contacting the cells with the ribonuclease inhibitor at day 4, 5, or 6 of maturation phase.

229. The method of any of embodiments 220-228, wherein the cell is in maturation phase.

230. The method of any of embodiments 220-229, which comprises contacting the cells with the ribonuclease inhibitor at a time when the cells comprise one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more) of the following properties:

i.a) 2-40%, 3-33%, 5-30%, 10-25%, or 15-20% of the cells in the population are enucleated;

i.b) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 2%, 3%, 4%, or 5% of the cells in the population are enucleated;

i.c) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 6%, 10%, 15%, 20%, or 25% of the cells in the population are enucleated;

i.d) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 30%, 35%, 40%, 45%, or 50% of the cells in the population are enucleated;

i.e) no more than 1%, 2%, 3%, 5%, 10%, 15%, or 20% of the cells in the population are enucleated;

i.f) no more than 25%, 30%, 35%, 40%, 45%, or 50% of the cells in the population are enucleated;

i.g) the population of cells has reached 6-70%, 10-60%, 20-50%, or 30-40% of maximal enucleation;

i.h) the population of cells has reached no more than 1%, 2%, 3%, 5%, 10%, 15%, or 20% of maximal enucleation;

i.i) the population of cells has reached no more than 25%, 30%, 35%, 40%, 45%, 50%, or 60% of maximal enucleation;

ii.a) the population of cells is fewer than 3, 2, or 1 population doubling from a plateau in cell division;

ii.b) the population of cells is capable of fewer than 3, 2, or 1 population doubling;

ii.c) the population will increase by no more than 1.5, 2, or 3 fold before the population reaches an enucleation level of at least 70% of cells in the population;

iii.a) at least 80%, 85%, 90%, 95%, or 99% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.b) at least 50%, 60%, 70%, 75%, or 79% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.c) 30-90%, 40-90%, 50-90%, 60-90%, or 70-90% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.d) at least 80%, 85%, 90%, 95%, or 99% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast);

iii.e) at least 50%, 60%, 70%, 75%, or 79% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast);

iii.f) 30-90%, 40-90%, 50-90%, 60-90%, or 70-90% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast);

iv.a) the population of cells has at least 60%, 70%, 80%, or 90% of maximal translational activity;

iv.b) the population of cells has at least 20%, 30%, 40%, or 50% of maximal translational activity;

iv.c) the population of cells has a translational activity of at least 600,000, 800,000, 1,000, 000, 1,200,000, 1,400,000, 1,600,000, 1,800,000, 2,000,000, 2,200,000, or 2,400,000, as measured by a BONCAT assay, e.g., by the translation assay of Example 10; or

iv. d) the population of cells has a translational activity of 600,000-2,400,000, 800,000-2,200,000, 1,000, 000-2,000,000, 1,200,000-1,800,000, or 1,400,000-1,600,000, as measured by a BONCAT assay, e.g., by the translation assay of Example 10.

231. The method of embodiment 230, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i and a property from ii.

232. The method of embodiment 230, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i and a property from iii.

233. The method of embodiment 230, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i and a property from iv.

234. The method of embodiment 230, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from ii and a property from iii.

235. The method of embodiment 230, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from ii and a property from iv.

236. The method of embodiment 230, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from iii and a property from iv.

237. The method of embodiment 230, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i, a property from ii, and a property from iii.

238. The method of embodiment 230, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i, a property from ii, and a property from iv.

239. The method of embodiment 230, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i, a property from iii, and a property from iv.

240. The method of embodiment 230, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from ii, a property from iii, and a property from iv.

241. The method of any of embodiments 220-240, which comprises contacting the cells with the ribonuclease inhibitor at a time when (e.g., by a flow cytometry assay, e.g., a flow cytometry assay of Example 10) the cells comprise one or more (e.g., 2, 3, 4, 5, or more) of the following properties:

84-99%, 85-95%, or about 90% of the cells in the population are GPA-positive;

at least 84%, 85%, 90%, 95%, or 99% of the cells in the population are GPA-positive;

54-99%, 55-98%, 60-95%, 65-90%, 70-85%, or 75-80% of the cells in the population are band3-positive;

at least 54%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% of the cells in the population are band3-positive;

96-100%, 97-99%, or about 98% of the cells in the population are alpha4 integrin-positive; or

at least 95%, 96%, 97%, 98%, or 99% of the cells in the population are alpha4 integrin-positive.

242. The method of any of embodiments 220-241, wherein the mRNA is in vitro transcribed mRNA.

243. The method of any of embodiments 220-242, wherein at least 80%, 85%, 90%, or 95% of the cells of the population are viable 5 days after the cells are contacted with the mRNA.

244. The method of any of embodiments 220-243, wherein at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the cells of the population are enucleated 5 days after the cells are contacted with the mRNA.

245. The method of any of embodiments 220-244, wherein the proportion of cells that are enucleated 5 days after the cells are contacted with the mRNA is at least 50%, 60%, 70%, 80%, 90%, or 95% of the proportion of cells that are enucleated in an otherwise similar population of cells not treated with the ribonuclease inhibitor.

246. The method of any of embodiments 220-245, wherein the population of cells comprises at least 1 x 10^6, 2 x 10^6, 5 x 10^6, 1 x 10^7, 2 x 10^7, 5 x 10^7, or 1 x 10^8 cells at the time the cells are contacted with the mRNA.

247. The method of any of embodiments 220-246, wherein the population of cells expands by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% within 5 days after the cells are contacted with the mRNA.

248. The method of any of embodiments 220-247, wherein at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population express the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA.

249. The method of any of embodiments 220-248, wherein at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population comprise at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA.

250. The method of any of embodiments 220-249, wherein the population of cells comprises at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% more, or at least 2-fold, 3-fold, 4-fold, or 5-fold more of the exogenous protein than an otherwise similar population of cells not treated with the ribonuclease inhibitor.

251. A reaction mixture comprising: i) an erythroid cell, ii) an mRNA comprising an exogenous protein and iii) a ribonuclease inhibitor.

252. The reaction mixture of embodiment 251, wherein the mRNA is inside the erythroid cell.

253. The reaction mixture of embodiment 251 or 252, which comprises a plurality of erythroid cells.

254. A method of assaying a reaction mixture comprising enucleated erythroid cells that comprise an exogenous protein for a ribonuclease inhibitor, comprising:

providing a reaction mixture comprising enucleated erythroid cells that comprise an exogenous protein,

assaying for the presence or level of a ribonuclease inhibitor, e.g., by ELISA, Western blot, or mass spectrometry, e.g., in an aliquot of the reaction mixture.

255. The method of embodiment 254, further comprising comparing the level of ribonuclease inhibitor to a reference value.

256. The method of embodiment 255, further comprising responsive to the comparison, one or more of:

classifying the population, e.g., as meeting a requirement or not meeting a requirement, e.g., wherein the requirement is met when the level of ribonuclease inhibitor is below the reference value,

classifying the population as suitable or not suitable for a subsequent processing step, e.g., when the population is suitable for a subsequent purification step when the level of ribonuclease inhibitor is above the reference value,

classifying the population as suitable or not suitable for use as a therapeutic, or

formulating or packaging the population, or an aliquot thereof, for therapeutic use, e.g., when the level of ribonuclease inhibitor is below the reference value.

257. The reaction mixture or method of any of embodiments 220-256, wherein the ribonuclease inhibitor is RNAsin Plus, Protector RNAse Inhibitor , or Ribonuclease Inhibitor Huma.

258. A method of making an erythroid cell comprising an mRNA that encodes an exogenous protein, comprising:

providing a reaction mixture comprising an erythroid cell and an mRNA encoding the exogenous protein, under conditions which inhibit protein degradation, e.g., by inclusion in the reaction mixture a proteasome inhibitor, and

maintaining the reaction mixture under conditions that allow uptake of the mRNA by the erythroid cell,

thereby making an erythroid cell comprising an mRNA encoding an exogenous protein.

259. The method of embodiment 258, comprising providing a population of erythroid cells and contacting the population with the mRNA encoding the exogenous protein.

260. The method of embodiment 258 or 259, wherein a plurality of erythroid cells of the population each takes up an mRNA encoding the exogenous protein.

261. The method of any of embodiments 258-260, wherein the cell or plurality of cells express the exogenous protein.

262. The method of any of embodiments 258-261, wherein the cell or plurality of cells comprise the exogenous protein.

263. The method of any of embodiments 258-262, which further comprises electroporating the cell or population of cells.

264. The method of any of embodiments 258-263, which further comprises contacting a population of erythroid cells with a proteasome inhibitor.

265. The method of any of embodiments 258-264, which comprises contacting the population of cells with the proteasome inhibitor before, during, or after contacting the cells with the mRNA, e.g., 0.5-2 days before or after contacting the cells with the mRNA.

266. The method of any of embodiments 258-265, which comprises contacting the cells with the proteasome inhibitor at day 4, 5, or 6 of maturation phase.

267. The method of any of embodiments 258-266, wherein the cell is in maturation phase.

268. The method of any of embodiments 258-267, which comprises contacting the cells with the proteasome inhibitor at a time when the cells comprise one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more) of the following properties:

i.a) 2-40%, 3-33%, 5-30%, 10-25%, or 15-20% of the cells in the population are enucleated;

i.b) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 2%, 3%, 4%, or 5% of the cells in the population are enucleated;

i.c) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 6%, 10%, 15%, 20%, or 25% of the cells in the population are enucleated;

i.d) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 30%, 35%, 40%, 45%, or 50% of the cells in the population are enucleated;

i.e) no more than 1%, 2%, 3%, 5%, 10%, 15%, or 20% of the cells in the population are enucleated;

i.f) no more than 25%, 30%, 35%, 40%, 45%, or 50% of the cells in the population are enucleated;

i.g) the population of cells has reached 6-70%, 10-60%, 20-50%, or 30-40% of maximal enucleation;

i.h) the population of cells has reached no more than 1%, 2%, 3%, 5%, 10%, 15%, or 20% of maximal enucleation;

i.i) the population of cells has reached no more than 25%, 30%, 35%, 40%, 45%, 50%, or 60% of maximal enucleation;

ii.a) the population of cells is fewer than 3, 2, or 1 population doubling from a plateau in cell division;

ii.b) the population of cells is capable of fewer than 3, 2, or 1 population doubling;

ii.c) the population will increase by no more than 1.5, 2, or 3 fold before the population reaches an enucleation level of at least 70% of cells in the population;

iii.a) at least 80%, 85%, 90%, 95%, or 99% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.b) at least 50%, 60%, 70%, 75%, or 79% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.c) 30-90%, 40-90%, 50-90%, 60-90%, or 70-90% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.d) at least 80%, 85%, 90%, 95%, or 99% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast);

iii.e) at least 50%, 60%, 70%, 75%, or 79% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast);

iii.f) 30-90%, 40-90%, 50-90%, 60-90%, or 70-90% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast);

iv.a) the population of cells has at least 60%, 70%, 80%, or 90% of maximal translational activity;

iv.b) the population of cells has at least 20%, 30%, 40%, or 50% of maximal translational activity;

iv.c) the population of cells has a translational activity of at least 600,000, 800,000, 1,000, 000, 1,200,000, 1,400,000, 1,600,000, 1,800,000, 2,000,000, 2,200,000, or 2,400,000, as measured by a BONCAT assay, e.g., by the translation assay of Example 10; or

iv. d) the population of cells has a translational activity of 600,000-2,400,000, 800,000-2,200,000, 1,000, 000-2,000,000, 1,200,000-1,800,000, or 1,400,000-1,600,000, as measured by a BONCAT assay, e.g., by the translation assay of Example 10.

269. The method of embodiment 268, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i and a property from ii.

270. The method of embodiment 268, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i and a property from iii.

271. The method of embodiment 268, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i and a property from iv.

272. The method of embodiment 268, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from ii and a property from iii.

273. The method of embodiment 268, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from ii and a property from iv.

274. The method of embodiment 268, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from iii and a property from iv.

275. The method of embodiment 268, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i, a property from ii, and a property from iii.

276. The method of embodiment 268, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i, a property from ii, and a property from iv.

277. The method of embodiment 268, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i, a property from iii, and a property from iv.

278. The method of embodiment 268, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from ii, a property from iii, and a property from iv.

279. The method of any of embodiments 258-278, which comprises contacting the cells with the proteasome inhibitor at a time when (e.g., by a flow cytometry assay, e.g., a flow cytometry

assay of Example 10) the cells comprise one or more (e.g., 2, 3, 4, 5, or more) of the following properties:

84-99%, 85-95%, or about 90% of the cells in the population are GPA-positive;

at least 84%, 85%, 90%, 95%, or 99% of the cells in the population are GPA-positive;

54-99%, 55-98%, 60-95%, 65-90%, 70-85%, or 75-80% of the cells in the population are band3-positive;

at least 54%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% of the cells in the population are band3-positive;

96-100%, 97-99%, or about 98% of the cells in the population are alpha4 integrin-positive; or

at least 95%, 96%, 97%, 98%, or 99% of the cells in the population are alpha4 integrin-positive.

280. The method of any of embodiments 258-279, wherein the mRNA is in vitro transcribed mRNA.

281. The method of any of embodiments 258-280, wherein at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population are viable 5 days after the cells are contacted with the mRNA.

282. The method of any of embodiments 258-281, wherein at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the cells of the population are enucleated 5 days after the cells are contacted with the mRNA.

283. The method of any of embodiments 258-282, wherein the proportion of cells that are enucleated 5 days after the cells are contacted with the mRNA is at least 50%, 60%, 70%, 80%, 90%, or 95% of the proportion of cells that are enucleated in an otherwise similar population of cells not treated with the proteasome inhibitor.

284. The method of any of embodiments 258-283, wherein the population of cells comprises at least 1 x 10^6, 2 x 10^6, 5 x 10^6, 1 x 10^7, 2 x 10^7, 5 x 10^7, or 1 x 10^8 cells at the time the cells are contacted with the mRNA.

285. The method of any of embodiments 258-284, wherein the population of cells expands by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% within 5 days after the cells are contacted with the mRNA.

286. The method of any of embodiments 258-285, wherein at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population express the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA.

287. The method of any of embodiments 258-286, wherein at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population comprise at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA.

288. The method of any of embodiments 258-287, wherein the population of cells comprises at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% more, or at least 2-fold, 3-fold, 4-fold, or 5-fold more of the exogenous protein than an otherwise similar population of cells not treated with the proteasome inhibitor.

289. A reaction mixture comprising: i) an erythroid cell, ii) an mRNA comprising an exogenous protein and iii) a proteasome inhibitor.

290. The reaction mixture of embodiment 289, wherein the mRNA is inside the erythroid cell.

291. The reaction mixture of embodiment 289 or 290, which comprises a plurality of erythroid cells.

292. A method of assaying a reaction mixture comprising enucleated erythroid cells that comprise an exogenous protein for a proteasome inhibitor, comprising:

providing a reaction mixture comprising enucleated erythroid cells that comprise an exogenous protein,

assaying for the presence or level of a proteasome inhibitor, e.g., by ELISA, Western blot, or mass spectrometry, e.g., in an aliquot of the reaction mixture.

293. The method of embodiment 292, further comprising comparing the level of proteasome inhibitor to a reference value.

294. The method of embodiment 293, further comprising, responsive to the comparison, one or more of:

classifying the population, e.g., as meeting a requirement or not meeting a requirement, e.g., wherein the requirement is met when the level of proteasome inhibitor is below the reference value,

classifying the population as suitable or not suitable for a subsequent processing step, e.g., when the population is suitable for a subsequent purification step when the level of proteasome inhibitor is above the reference value,

classifying the population as suitable or not suitable for use as a therapeutic, or

formulating or packaging the population, or an aliquot thereof, for therapeutic use, e.g., when the level of proteasome inhibitor is below the reference value.

295. The reaction mixture or method of any of embodiments 258-294, wherein the proteasome inhibitor is a 20S proteasome inhibitor, e.g., MG-132 or carfilzomib, or a 26S proteasome inhibitor, e.g., bortezomib.

296. A method of making an erythroid cell comprising an mRNA encoding a first exogenous protein and a second exogenous protein, comprising:

a) providing an erythroid cell, e.g., in maturation phase, and

b) contacting the erythroid cell with an mRNA encoding the first exogenous protein and a second mRNA encoding the second exogenous protein, under conditions that allow uptake of the first mRNA and second mRNA by the erythroid cell,

thereby making an erythroid cell comprising the first mRNA and the second mRNA.

297. The method of embodiment 296, wherein the erythroid cell comprises at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the first exogenous protein and the second exogenous protein, e.g., 5 days after the contacting with the mRNA.

298. A method of producing a population of erythroid cells expressing a first exogenous protein and a second exogenous protein, comprising:

a) providing a population of erythroid cells, e.g., in maturation phase, and

b) contacting the population of erythroid cells with a first mRNA encoding a first protein and a second mRNA encoding a second protein,

thereby making an erythroid cell comprising an mRNA encoding an exogenous protein

wherein at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of cells in the population comprise both of the first mRNA and the second mRNA.

299. The method of embodiment 298, wherein the population of erythroid cells comprises an average of at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the first exogenous protein and the second exogenous protein per cell, e.g., 5 days after the contacting with the mRNA.

300. The method of any of embodiments 296-299, wherein the contacting comprises performing electroporation.

301. The method of any of embodiments 298-300, wherein the population of cells comprise the first exogenous protein and the second exogenous protein in at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of cells for at least 5 days after the cells were contacted with the first and second mRNAs.

302. A population of erythroid cells wherein at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of cells in the population express a first exogenous protein and a second exogenous protein, wherein the population was not made by contacting the cells with DNA encoding the first or second exogenous protein.

303. A method of producing a plurality of erythroid cells comprising a predetermined number of copies of an exogenous protein per cell, comprising contacting the population with a predetermined amount of mRNA encoding the exogenous protein, thereby making the erythroid cell comprising the predetermined amount of the exogenous protein.

304. The method of embodiment 303, further comprising evaluating one or more of the plurality of erythroid cells (e.g., enucleated erythroid cells) to determine the amount of the exogenous protein.

305. A method of evaluating the amount of an exogenous protein in a sample of erythroid cells, e.g., enucleated erythroid cells comprising:

providing a plurality of erythroid cells comprising a predetermined number of copies of an exogenous protein per cell, which was made by contacting the population with a predetermined amount of mRNA encoding the exogenous protein, and

determining the amount of the exogenous protein in the plurality of erythroid cells.

306. The method of embodiment any of embodiments 303-305, wherein:

contacting the cell population with 0.6 ±20% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 1,000,000 ±20% copies of the exogenous protein per cell,

contacting the cell population with 0.4 ±20% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 870,000 ±20% copies of the exogenous protein per cell,

contacting the cell population with 0.2 ±20% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 610,000 ±20% copies of the exogenous protein per cell,

contacting the cell population with 0.1±20% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 270,000±20% copies of the exogenous protein per cell,

contacting the cell population with 0.05±20% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 100,000 ±20% copies of the exogenous protein per cell, or

contacting the cell population with 0.025 ±20% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 43,000 ±20% copies of the exogenous protein per cell.

307. The method of any of embodiments 303-306, wherein at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population express the exogenous protein 1 day after the cells are contacted with the exogenous protein.

EXAMPLES

Example 1: Methods of delivering exogenous modified or unmodified RNA

For lentivirally transduced K562 cells the expression of an epitope tag (HA tag) contained on the transgene is inversely correlated to the provirus length, with an approximately 1-log decrease in percent of cells expressing the HA tag for provirus constructs larger than approximately 6 kb (see Figure 1). While not intending to be bound by any particular theory, it is believed that the reason for the decrease in transduction efficiency has to do with the reduced packaging efficiency of longer provirus sequences within lentiviruses. A set of lentivirus constructs of provirus lengths ranging from 3.6 kb to 8.2 kb were tested (see Figure 2). The number of lentivirus particles produced was quantified using the number of p24 capsid proteins measured by ELISA. The number of copies of provirus RNA was measured by quantitative polymerase chain reaction (qPCR). Normalization provides a quantification of RNA copies per microgram p24 capsid protein as a function of provirus length. In the experiments, for provirus length < 5kb about $3x10^9$ RNA copies per microgram p24 could be seen. For constructs > 6 kb no virus preparation exhibited more than about $8x10^8$ RNA copies per microgram p24 capsid. A size-dependent difference between RNA-containing and RNA-deficient virus preparations can be observed leading to a reduction in transduction efficiency.

Electroporation by a single pulse of 260V/150μF of K562 cells and cultured erythroid cells (from primary cells) with mRNA encoding for green fluorescent protein (GFP) was performed (see Figure 3). Successful gene transfer was measured by reading the fluorescence

from the GFP, which requires that the mRNA enter the cell and then be translated into protein. The conditions from the literature that lead to successful electroporation of K562 cells (Van Tendeloo et al., Blood 2001 98(1):49-56) are insufficient for the effective delivery of exogenous nucleic acids to cultured erythroid cells (derived from primary progenitors). More than 50 different conditions for electroporation of cultured erythroid cells from primary progenitors were tested. Transfection efficiencies generally ranged from 0.1% transfected cells to more than 85% transfected cells (see Figures 4A-4C). Figures 4A-4C show the translation of GFP from mRNA following electroporation of cultured erythroid cells from primary progenitors at day 8 of differentiation for 12 different conditions. Viability was measured using LIVE/DEAD stain from Life Technologies, in which cells that were negative for the stain were considered viable. Condition 1 corresponds to the untransfected control (0.21% GFP, 97.39% viability). Depending on the electroporation conditions used, cells had very good uptake of mRNA (86.9%) and high viability (92.6%), e.g. condition 2, or poor uptake of mRNA (30.9%) and poor viability (42.7%), e.g. condition 9.

As the cells continue to differentiate, different electroporation conditions are required to achieve good transgene uptake and expression while maintaining high viability. Greater than 50 conditions were tested on cells over the course of an approximately 20 day differentiation culture to identify conditions that were conducive to good transfection and good viability. Figure 5 shows the successful transfection of cells with GFP mRNA by electroporation at three different time points – day 8, day 13, and day 15. Suitable conditions are summarized in Tables 5 to 7. Cultured erythroid cells were also transfected with GFP mRNA by electroporation on day 10 and day 12 of differentiation, resulting in GFP expression (data not shown).

It was also observed that electroporation under the conditions disclosed herein of erythroid cells cultured from primary progenitors did not appear to damage the cells' ability to terminally differentiate. Cells that had been electroporated once were re-electroporated and again successfully took up and translated the transgene. Figure 6 shows a population of erythroid cells cultured from primary progenitors that were electroporated at day 9, allowed to divide for four days during which the amount of GFP fluorescence decreased – likely because of dilution of the mRNA and protein through cell division – and then re-electroporated at day 13.

Cultured erythroid cells were electroporated with GFP mRNA on day 4 of differentiation, which is during the expansion phase where the cells are relatively undifferentiated. On day 8 of

differentiation, the cells showed GFP fluorescence and high viability by 7AAD staining, as shown in Table 8. In Table 8, P1 indicates the percentage of the main population that constitutes cells (e.g., high P1 values mean low levels of debris); % GFP indicates the percent of cells in P1 that show GFP fluorescence, MFI is the mean fluorescent intensity of the GFP+ cells, and %AAD- indicates the percent of cells that are AAD negative, where viable cells are AAD negative.

Example 2: ELISA

P24 protein was quantified using a commercial kit (Clontech) following manufacturer's protocol. Briefly, viral supernatants were dispensed into tubes with 20 uL lysis buffer and incubated at 37C for 60 minutes, then transferred to a microtiter plate. The microtiter plate was washed and incubated with 100 µl of Anti-p24 (Biotin conjugate) detector antibody at 37C for 60 minutes. Following a wash, the plate was incubated with 100 µl of Streptavidin-HRP conjugate at room temperature for 30 minutes, then washed again. 12. Substrate Solution was added to the plate and incubated at room temperature (18–25°C) for 20 (±2) minutes. The reaction was topped with stop solution, and colorimetric readout detected by absorbance at 450 nm.

Example 3: qPCR

Viral RNA copies were quantified using a commercial lentivector qRT-PCR kit (Clontech) following manufacturer's protocol. Briefly, an RNA virus purification kit was used to extract RNA from lentiviral supernatant. The PCR reaction was performed with standard lentivirus primers (forward and reverse) that recognize conserved sequences on the viral genome and are not dependent on the specific transgene encoded by the vector. The RT reaction was performed with a 42C 5 min incubation followed by a 95 °C 10 sec incubation, followed by 40 cycles of 95C for 5 sec and 60C for 30 sec. The instrument used was a Life Technologies QuantStudio.

Example 4: Production of mRNA by in vitro transcription

Kits for in vitro production of mRNA are available commercially, e.g., from Life Technologies MAxiscript T7 kit. Briefly, a gene of interest is cloned into an appropriate T7 promoter-containing plasmid DNA by standard molecular biology techniques. The transcription reaction is set up with 1 ug DNA template, 2 uL 10x transcription buffer, 1 uL each of 10 mM ATP, CTP, GTP, and UTP, 2 uL of T7 polymerase enzyme mix, in a total volume of 20 uL. The reaction is mixed thoroughly and incubated for 1 hr at 37C. To remove contaminating residual

plasmid DNA, 1 uL turbo DNAse is added and the reaction incubated for 15 minutes at 37C. The reaction is stopped by the addition of 1 uL 0.5 M EDTA. The transcript is purified by gel electrophoresis or spin column purification.

Example 5: Electroporation

Cells are washed in RPMI buffer, loaded into a Life Technologies Neon electroporation instrument at a density of 1 x 10^7 cells/mL in a total volume of 10 uL, and electroporated with the following conditions: 1 pulse of 1000 V, 50 ms pulse width.

Example 6: Electroporation with chemically modified mRNA

Chemically modified mRNA encoding GFP was purchased from TriLink. The RNA contains pseudo-uridine and 5-methyl cytosine. Differentiating erythroid cells were electroporated at day 4, 8, 10, or 12 of differentiation. On all days of differentiation tested, and under different electroporation conditions tested, GFP fluorescence was observed. Table 9 indicates the GFP fluorescence levels observed when cells were electroporated on day 4 and observed on day 8. Table 10 indicates GFP fluorescence levels observed when cells were electroporated on day 12 and observed on day 15. GFP fluorescence was also observed in cells electroporated at day 8 or 10 of differentiation (data not shown).

Cell viability and proliferation ability were measured in electroporated cells, using trypan blue staining. The cells were electroporated at day 8 of differentiation with unmodified GFP mRNA or TriLink chemically modified RNA comprising pseudo-uridine and 5-methyl cytosine. On day 9, GFP fluorescence was observed in the cells receiving unmodified or modified RNA (data not shown). Also on day 9, the total number of cells, number of live cells, and cell viability were measured. In the samples electroporated with unmodified mRNA, the number of live cells was lower than the number of live cells in the control cells that were electroporated without adding exogenous nucleic acid (see Table 11). This decline was partially reversed when modified RNA was used (Table 11). This indicates that electroporation with unmodified RNA may reduce cell growth or viability, and use of modified RNA can at least partially rescue growth or viability.

Example 7: Heterologous untranslated regions

Erythroid cells were electroporated with in vitro transcribed, GFP mRNA having a hemoglobin 3' UTR sequence appended ("Hemo-GFP"). The mRNA was not chemically modified. The cells were then assayed for GFP fluorescence by flow cytometry two days after

electroporation. 59.7% of the cells were GFP-positive. The mean fluorescence intensity of the GFP-positive cells was 35069 units.

Example 8: mRNA electroporation during maturation phase

As illustrated in Figure 7A, red blood cell differentiation can be divided into three phases: expansion (days 0-5 of expansion, which correspond to days 0-5 overall), differentiation (days 1-9 of differentiation, which correspond to days 6-14 overall), and maturation (days 1-14 of maturation, which correspond to days 15-28 overall). Expansion describes the phase of hematopoietic progenitor cell isolation and expansion in a non-differentiating environment, in order to amplify early stage cultures to meet clinical dose requirements. Differentiation describes the use of growth factors and media additives to induce erythropoiesis and specialize for red blood cell function. Maturation refers to a final stage in which red blood cells first lose their nucleus and subsequently their mitochondria and ribosome content. The mature red blood cell does not have the capacity for new mRNA synthesis or protein translation.

Red blood cell differentiation was performed in vitro, and the cells were electroporated with GFP mRNA at different timepoints. When the cells were electroporated at differentiation day 9 (overall day 14), GFP expression was observed initially but declined over the course of the 9-day experiment (Figure 7B). When cells were electroporated on maturation day 7 (overall day 21), GFP expression was prolonged throughout the course of the 9-day experiment (Figure 7C). Under four different electroporation protocols (P1-P4) the result was similar, indicating that this effect is relatively independent of electroporation conditions.

It was surprising that electroporation at such late stages worked as well as it did. As cited by Steinberg (Steinberg, M., Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management, Cambridge University Press, 2001) The adult red cell is organized to carry the synthesized hemoglobin for its role in gaseous transport; the nucleus, the capacity for protein synthesis, and the ability to diversify its function have been cast off for the ultimate purpose of hemoglobin transport via biologically economical means." The art generally regarded maturation as a phase when erythroid cells are enucleated and shed ribosomes and mitochondria. Shedding ribosomes leads to the expectation that maturation phase erythroid cells translate poorly and therefore should be incapable of new protein synthesis.

Thus, it was surprising that maturation phase erythroid cells could translate a transgenic mRNA at least as well as a differentiation phase erythroid cell, and even more surprising that the

maturation phase erythroid cell produced more sustained level of transgenic protein than the differentiation phase erythroid cell. This identifies a unique stage of erythroid development, contrary to traditional models, in which new protein synthesis from exogenously provided RNA can be achieved in enucleated red blood cells. This identifies hitherto unknown pathways for achieving stable protein production in late stage red blood cell products.

Example 9: Timing of electroporation

Several different timepoints were tested for electroporating an mRNA encoding a reporter protein (GFP) into a population of erythroid cells under maturation conditions. Specifically, electroporation was tested at days 4, 5, 6, and 7 of maturation. The cells were assayed for GFP expression by flow cytometry at every 24 hours for at least 6 days after electroporation. Suitable electroporation conditions are described, e.g., in Example 1 herein and in International Application WO2016/183482, which is herein incorporated by reference in its entirety.

As shown in Fig. 8A, cells electroporated at all timepoints gave prolonged GFP expression. However, cells electroporated at days M4 and M5 gave a higher percentage of cells expressing GFP than cells electroporated at M6 or M7. This experiment indicates a window of erythroid cell maturation that is particularly amenable to expression of a transgene. Fig. 8B shows that GFP levels in the population decline somewhat in cells transfected at M4 or M5 over the time course; however GFP expression in these cells is still higher than that in control cells and cells electroporated at later timepoints.

While not wishing to be bound by theory, the window may indicate a timepoint that is early in maturation enough that the cell's translation machinery has not yet been lost, while simultaneously being late enough in maturation that the exogenous mRNA and encoded protein do not get unduly diluted by subsequent cell division. This window was further characterized as described in Example 10.

Example 10: Characteristics of maturing erythroid cells

Next, maturing erythroid cells were characterized at several timepoints for their translation activity and enucleation level. Translational activity was measured by biorthogonal noncanonical amino acid tagging, or BONCAT. Suitable BONCAT assays are described, e.g., in

Hatzenpichler et al., "In situ visualization of newly synthesized proteins in environmental microbes using amino acid tagging and click chemistry" Environmental Microbiology (2014) 16(8), 2568–2590. This assay is based on the in vivo incorporation of a surrogate for L-methionine, the non-canonical amino acid L-azidohomoalanine (AHA), following fluorescent labeling of incorporated AHA cellular proteins by Click Chemistry. The protocol has been modified and optimized for mammalian primary cells particularly human erythroid progenitors by increasing the AHA concentration from 1mM to 2mM, optimized the incubation time to 3h, and dibenzocyclooctyne group (DBCO) has been used which allows Copper-free Click Chemistry to be analyzed by gel electrophoresis and infrared imaging. A population of erythroid cells was exposed to expansion, differentiation, and maturation conditions, and samples of $3x10^6$ cells were collected on days M3, M5, M7, M9, M11, M15, and M16. As shown in Fig. 9, translational activity of the cells declined dramatically over the time course, indicating that the erythroid cells were losing translation machinery. Over the same time course, the proportion of enucleated cells in the population rose dramatically.

Cell surface markers were also assayed in erythroid cells at different stages of maturation, by flow cytometry. As shown in Table 13, the percentage of GPA-positive cells and Band3-positive cells rose from during maturation, and the percentage of Alpha4 integrin-positive remained high throughout the time course.

Example 11: Ribonuclease inhibitors increase protein expression in electroporated erythroid cells

This experiment demonstrates that exposing erythroid cells to ribonuclease inhibitors increases expression of a transgene.

Erythroid cells were differentiated, exposed to maturation conditions, and electroporated at day M4 with mRNA encoding a reporter gene (mCherry). 2×10^6 cells were treated with RNasin before the mRNA was added to the cells at a level of 0.5 U/uL, 1 U/uL, or 2 U/ul, or no RNasin as a control. A non-electroporated control was also included. The cells were assayed at days M5, M7, M9, and M11. As shown in Figure 10, the percentage of cells expressing mCherry was higher in cells treated with RNasin than in cells without RNasin, especially at the M11 timepoint. RNasin treatment did not negatively impact cell viability or enucleation (data not shown).

Example 12: Proteasome inhibitors increase protein expression in electroporated erythroid cells

This experiment demonstrates that exposing erythroid cells to protease inhibitors increases expression of a transgene.

Erythroid cells were differentiated, exposed to maturation conditions, and electroporated at day M5 with mRNA encoding a reporter gene (GFP). Cells were treated with a proteasome inhibitor selected from MG-132, bortezomib, and carfilzomib, at day M4, M5, or M6. All cell samples resulted in a high percentage of GFP-positive cells, over 75%, when assayed at M7, M9, and M11 (data not shown). As shown in Fig. 11, treatment with the 20S proteasome inhibitors, MG-132 or bortezomib, before electroporation, resulted in increased effective expression of GFP at one or more timepoints. The bortezomib treatment resulted in a 4-fold increase in effective expression of GFP compared to cells not treated with a proteasome inhibitor. Treatment with the 20S proteasome inhibitors before electroporation also resulted in normal enucleation (data not shown).

Example 13: Co-expression of two or more RNAs

This Example demonstrates co-expression of two or more mRNAs in erythroid cells.

First, erythroid cells were electroporated at day M5 with EGFP mRNA alone (Table 14, first data column), mCherry mRNA alone (Table 14, second data column), or both mRNAs (Table 14, third data column). EGFP and mCherry fluorescence was assayed by flow cytometry on days M6, M11, M18, and M18. The percentage of cells expressing both). EGFP and mCherry was consistently high across timepoints (66.05%-86.55%) and comparable to the percent of cells fluorescing after electroporation with just one of the mRNAs. This experiment indicates that it is possible to achieve uniform expression of two mRNAs simultaneously.

Expression levels were also assayed. At day M13, the effective expression of mCherry in cells electroporated with mCherry mRNA only was 117, and the effective expression of mCherry in cells electroporated with both mCherry mRNA and EGFP mRNA was 85. The effective expression of EGFP in cells electroporated with EGFP mRNA only was 219, and the effective expression of EGFP in cells electroporated with both mCherry mRNA and EGFP mRNA was 201. Thus, expression levels were similar in cells electroporated with one or two mRNAs.

Viability was no lower in cells co-electroporated with two mRNAs than in cells electroporated with either mRNA alone (data not shown).

Another pair of mRNAs, encoding HA-tagged m4-1BBL and FLAG-tagged Avelumab were co-expressed in erythroid cells. RNA was added to $25x10^6$ erythroid cells at differentiation day 6 (D6) or differentiation day 7 (D7), at a concentration of 0.6 or 0.8 mg/ml mRNA. The exogenous proteins were detected by flow cytometry using an anti-HA antibody and an anti-FLAG antibody. As shown in Table 15, co-expression of the proteins was achieved in 58.5% of cells, a number comparable to the number of cells that expressed either protein alone in samples electroporated with only one of the mRNAs.

Example 14: Dose-expression studies

This experiment demonstrates that a predetermined amount of an exogenous protein can be produced by contacting a population of erythroid cells with a predetermined amount of mRNA encoding the exogenous protein.

5×10^6 cells at day 4 of maturation were contacted with different amounts of mRNA (between 0.0025 and 0.6 ug RNA per sample) and electroporated. Protein expression was assayed 24 hours after electroporation. The exogenous protein was quantified by flow cytometry using an anti-HA antibody. The average number of proteins per cell was calculated and is shown in Table 16. The percent of cells expressing the exogenous protein is also shown in Table 16. Notably, at all mRNA levels tested, the percent of cells expressing the exogenous protein is high. However, the number of copies per cell rises roughly linearly with the amount of mRNA used. Thus, the amount of protein expression desired can be obtained by selecting an appropriate of mRNA, while maintaining uniform expression across the population of cells.

Example 15: Expression from modified RNAs

Modified mRNA was produced, comprising one or more of a 5' cap (ARCA), polyA tail, and pseudouridine. The mRNA comprises an IRES to promote translation, an HA-encoding region to facilitate detection, and a region encoding a fusion of GFP and PAL (phenylalanine ammonia lyase). The mRNA was introduced into erythroid cells by electroporation at day M4 and was analyzed at days M5 (24 hours later), M6, M7, and M10. GFP expression was measured by flow cytometry. As shown in Fig. 12, cells expressing pseudouridine mRNA had a higher percentage of GFP-positive cells than cells expressing completely unmodified RNA. Addition of a polyA tail and cap increased the percentage of GFP-positive cells further. Finally,

the percentage of cells showing expression of the GFP reporter was highest in the cells contacted with mRNA having a cap, poly-A tail, and pseudouridine incorporation.

TABLES

Table 1. Modified nucleotides

5-aza-uridine	N2-methyl-6-thio-guanosine
2-thio-5-aza-midine	N2,N2-dimethyl-6-thio-guanosine
2-thiouridine	pyridin-4-one ribonucleoside
4-thio-pseudouridine	2-thio-5-aza-uridine
2-thio-pseudouridine	2-thiomidine
5-hydroxyuridine	4-thio-pseudomidine
3-methyluridine	2-thio-pseudowidine
5-carboxymethyl-uridine	3-methylmidine
1-carboxymethyl-pseudouridine	1-propynyl-pseudomidine
5-propynyl-uridine	1-methyl-1-deaza-pseudomidine
1-propynyl-pseudouridine	2-thio-1-methyl-1-deaza-pseudouridine
5-taurinomethyluridine	4-methoxy-pseudomidine
1-taurinomethyl-pseudouridine	5'-O-(1-Thiophosphate)-Adenosine
5-taurinomethyl-2-thio-uridine	5'-O-(1-Thiophosphate)-Cytidine
1-taurinomethyl-4-thio-uridine	5'-O-(1-thiophosphate)-Guanosine
5-methyl-uridine	5'-O-(1-Thiophophate)-Uridine
1-methyl-pseudouridine	5'-O-(1-Thiophosphate)-Pseudouridine
4-thio-1-methyl-pseudouridine	2'-O-methyl-Adenosine
2-thio-1-methyl-pseudouridine	2'-O-methyl-Cytidine
1-methyl-1-deaza-pseudouridine	2'-O-methyl-Guanosine
2-thio-1-methyl-1-deaza-pseudomidine	2'-O-methyl-Uridine
dihydrouridine	2'-O-methyl-Pseudouridine
dihydropseudouridine	2'-O-methyl-Inosine
2-thio-dihydromidine	2-methyladenosine
2-thio-dihydropseudouridine	2-methylthio-N6-methyladenosine
2-methoxyuridine	2-methylthio-N6 isopentenyladenosine
2-methoxy-4-thio-uridine	2-methylthio-N6-(cis-hydroxyisopentenyl)adenosine
4-methoxy-pseudouridine	
4-methoxy-2-thio-pseudouridine	N6-methyl-N6-threonylcarbamoyladenosine
5-aza-cytidine	N6-hydroxynorvalylcarbamoyladenosine
pseudoisocytidine	2-methylthio-N6-hydroxynorvalyl carbamoyladenosine
3-methyl-cytidine	
N4-acetylcytidine	2'-O-ribosyladenosine (phosphate)
5-formylcytidine	1,2'-O-dimethylinosine
N4-methylcytidine	5,2'-O-dimethylcytidine
5-hydroxymethylcytidine	N4-acetyl-2'-O-methylcytidine
1-methyl-pseudoisocytidine	Lysidine
pyrrolo-cytidine	7-methylguanosine
pyrrolo-pseudoisocytidine	N2,2'-O-dimethylguanosine
2-thio-cytidine	N2,N2,2'-O-trimethylguanosine
2-thio-5-methyl-cytidine	2'-O-ribosylguanosine (phosphate)
4-thio-pseudoisocytidine	Wybutosine
4-thio-1-methyl-pseudoisocytidine	Peroxywybutosine

4-thio-1-methyl-1-deaza-pseudoisocytidine	Hydroxywybutosine
1-methyl-1-deaza-pseudoisocytidine	undermodified hydroxywybutosine
zebularine	methylwyosine
5-aza-zebularine	queuosine
5-methyl-zebularine	epoxyqueuosine
5-aza-2-thio-zebularine	galactosyl-queuosine
2-thio-zebularine	mannosyl-queuosine
2-methoxy-cytidine	7-cyano-7-deazaguanosine
2-methoxy-5-methyl-cytidine	7-aminomethyl-7-deazaguanosine
4-methoxy-pseudoisocytidine	archaeosine
4-methoxy-1-methyl-pseudoisocytidine	5,2'-O-dimethyluridine
2-aminopurine	4-thiouridine
2,6-diaminopurine	5-methyl-2-thiouridine
7-deaza-adenine	2-thio-2'-O-methyluridine
7-deaza-8-aza-adenine	3-(3-amino-3-carboxypropyl)uridine
7-deaza-2-aminopurine	5-methoxyuridine
7-deaza-8-aza-2-aminopurine	uridine 5-oxyacetic acid
7-deaza-2,6- diaminopurine	uridine 5-oxyacetic acid methyl ester
7-deaza-8-aza-2,6-diarninopurine	5-(carboxyhydroxymethyl)uridine)
1-methyladenosine	5-(carboxyhydroxymethyl)uridine methyl ester
N6-isopentenyladenosine	5-methoxycarbonylmethyluridine
N6-(cis-hydroxyisopentenyl)adenosine	5-methoxycarbonylmethyl-2'-O-methyluridine
2-methylthio-N6-(cis-hydroxyisopentenyl) adenosine	5-methoxycarbonylmethyl-2-thiouridine
	5-aminomethyl-2-thiouridine
N6-glycinylcarbamoyladenosine	5-methylaminomethyluridine
N6-threonylcarbamoyladenosine	5-methylaminomethyl-2-thiouridine
2-methylthio-N6-threonyl carbamoyladenosine	5-methylaminomethyl-2-selenouridine
	5-carbamoylmethyluridine
N6,N6-dimethyladenosine	5-carbamoylmethyl-2'-O-methyluridine
7-methyladenine	5-carboxymethylaminomethyluridine
2-methylthio-adenine	5-carboxymethylaminomethyl-2'-O-methyluridine
2-methoxy-adenine	
inosine	5-carboxymethylaminomethyl-2-thiouridine
1-methyl-inosine	N4,2'-O-dimethylcytidine
wyosine	5-carboxymethyluridine
wybutosine	N6,2'-O-dimethyladenosine
7-deaza-guanosine	N,N6,O-2'-trimethyladenosine
7-deaza-8-aza-guanosine	N2,7-dimethylguanosine
6-thio-guanosine	N2,N2,7-trimethylguanosine
6-thio-7-deaza-guanosine	3,2'-O-dimethyluridine
6-thio-7-deaza-8-aza-guanosine	5-methyldihydrouridine
7-methyl-guanosine	5-formyl-2'-O-methylcytidine
6-thio-7-methyl-guanosine	1,2'-O-dimethylguanosine
7-methylinosine	4-demethylwyosine
6-methoxy-guanosine	Isowyosine
1-methylguanosine	N6-acetyladenosine

N2-methylguanosine N2,N2-dimethylguanosine 8-oxo-guanosine 7-methyl-8-oxo-guanosine 1-methyl-6-thio-guanosine	

Table 2. Backbone modifications

2'-O-Methyl backbone
Peptide Nucleic Acid (PNA) backbone
phosphorothioate backbone
morpholino backbone
carbamate backbone
siloxane backbone
sulfide backbone
sulfoxide backbone
sulfone backbone
formacetyl backbone
thioformacetyl backbone
methyleneformacetyl backbone
riboacetyl backbone
alkene containing backbone
sulfamate backbone
sulfonate backbone
sulfonamide backbone
methyleneimino backbone
methylenehydrazino backbone
amide backbone

Table 3. Modified caps

m7GpppA
m7GpppC
m2,7GpppG
m2,2,7GpppG
m7Gpppm7G
m7,2'OmeGpppG
m72'dGpppG
m7,3'OmeGpppG
m7,3'dGpppG
GppppG
m7GppppG
m7GppppA
m7GppppC
m2,7GppppG
m2,2,7GppppG

m7Gppppm7G m7,2'OmeGppppG m72'dGppppG m7,3'OmeGppppG m7,3'dGppppG

Table 4. Selected Diseases, Receivers and Targets

Category	Disease	Exogenous polypeptide	Target
Amyloidoses	AA Amyloidosis	an antibody-like binder to serum amyloid A protein or serum amyloid P component	Serum amyloid A protein and amyloid placques
Amyloidoses	beta2 microglobulin amyloidosis	an antibody-like binder to beta-2 microglobulin or serum amyloid P component	Beta2 microglobulin or amyloid placques
Amyloidoses	Light chain amyloidosis	an antibody-like binder to light chain, serum amyloid P component	Antibody light chain or amyloid placques
Cell clearance	Cancer	an antibody-like binder to CD44	a circulating tumor cell
Cell clearance	Cancer	an antibody-like binder to EpCam	a circulating tumor cell
Cell clearance	Cancer	an antibody-like binder to Her2	a circulating tumor cell
Cell clearance	Cancer	an antibody-like binder to EGFR	a circulating tumor cell
Cell clearance	Cancer (B cell)	an antibody-like binder to CD20	a cancerous B cell
Cell clearance	Cancer (B cell)	an antibody-like binder to CD19	a cancerous B cell
Clearance Ab	Antiphospholipid syndrome	beta2-glycoprotein-1	pathogenic self-antibody against beta2-glycoprotein-1
Clearance Ab	Catastrophic antiphospholipid syndrome	beta2-glycoprotein-1	pathogenic self-antibody against beta2-glycoprotein-1
Clearance Ab	Cold agglutinin disease	I/i antigen	Pathogenic self-antibody against I/i antigen
Clearance Ab	Goodpasture syndrome	a3 NC1 domain of collagen (IV)	pathogenic self-antibody against a3 NC1 domain of Collagen (IV)
Clearance Ab	Immune thrombocytopenia purpura	Platelet Glycoproteins (Ib-IX, IIb-IIIa, IV, Ia-IIa)	pathogenic self-antibody against platelet glycoprotein
Clearance Ab	Membranous Nephropathy	Phospholipase A2 receptor	pathogenic self-antibody against phospholipase A2 receptor
Clearance Ab	Warm antibody hemolytic anemia	Glycophorin A, glycophorin B, and/or glycophorin C, Rh antigen	pathogenic self-antibody against glycophorins and/or Rh

			antigen
Complement	Age-related macular degeneration	a suitable complement regulatory protein	active complement
Complement	Atypical hemolytic uremic syndrome	complement factor H, or a suitable complement regulatory protein	active complement
Complement	Autoimmune hemolytic anemia	a suitable complement regulatory molecule	active complement
Complement	Complement Factor I deficiency	Complement factor I, a suitable complement regulatory protein	active complement
Complement	Non-alcoholic steatohepatitis	a suitable complement regulatory molecule	active complement
Complement	Paroxysmal nocturnal hemoglobinuria	a suitable complement regulatory protein	active complement
Enzyme	3-methylcrotonyl-CoA carboxylase deficiency	3-methylcrotonyl-CoA carboxylase	3-hydroxyvalerylcarnitine, 3-methylcrotonylglycine (3-MCG) and 3-hydroxyisovaleric acid (3-HIVA)
Enzyme	Acute Intermittent Porphyria	Porphobilinogen deaminase	Porphobilinogen
Enzyme	Acute lymphoblastic leukemia	Asparaginase	Asparagine
Enzyme	Acute lymphocytic leukemia, acute myeloid leukemia	Asparaginase	Asparagine
Enzyme	Acute myeloblastic leukemia	Asparaginase	Asparagine
Enzyme	Adenine phosphoribosyltransferase deficiency	adenine phosphoribosyltransferase	Insoluble purine 2,8-dihydroxyadenine
Enzyme	Adenosine deaminase deficiency	Adenosine deaminase	Adenosine
Enzyme	Afibrinogenomia	FI	enzyme replacement
Enzyme	Alcohol poisoning	Alcohol dehydrogenase/oxidase	Ethanol
Enzyme	Alexander's disease	FVII	enzyme replacement
Enzyme	Alkaptonuria	homogentisate oxidase	homogentisate
Enzyme	Argininemia	Ammonia monooxygenase	ammonia
Enzyme	argininosuccinate aciduria	Ammonia monooxygenase	ammonia
Enzyme	citrullinemia type I	Ammonia monooxygenase	ammonia
Enzyme	Citrullinemia type II	Ammonia monooxygenase	ammonia
Enzyme	Complete LCAT deficiency, Fish-eye disease, atherosclerosis, hypercholesterolemia	Lecithin-cholesterol acyltransferase (LCAT)	Cholesterol
Enzyme	Cyanide poisoning	Thiosulfate-cyanide	Cyanide

		sulfurtransferase	
Enzyme	Diabetes	Hexokinase, glucokinase	Glucose
Enzyme	Factor II Deficiency	FII	enzyme replacement
Enzyme	Familial hyperarginemia	Arginase	Arginine
Enzyme	Fibrin Stabilizing factor Def.	FXIII	enzyme replacement
Enzyme	Glutaric acidemia type I	lysine oxidase	3-hydroxyglutaric and glutaric acid (C5-DC), lysine
Enzyme	Gout	Uricase	Uric Acid
Enzyme	Gout - hyperuricemia	Uricase	Uric acid (Urate crystals)
Enzyme	Hageman Def.	FXII	enzyme replacement
Enzyme	Hemolytic anemia due to pyrimidine 5' nucleotidase deficiency	pyrimidine 5' nucleotidase	pyrimidines
Enzyme	Hemophilia A	Factor VIII	Thrombin (factor II a) or Factor X
Enzyme	Hemophilia B	Factor IX	Factor XIa or Factor X
Enzyme	Hemophilia C	FXI	enzyme replacement
Enzyme	Hepatocellular carcinoma, melanoma	Arginine deiminase	Arginine
Enzyme	Homocystinuria	Cystathionine B synthase	homocysteine
Enzyme	hyperammonemia/ornithinemia/citrullinemia (ornithine transporter defect)	Ammonia monooxygenase	Ammonia
Enzyme	Isovaleric acidemia	Leucine metabolizing enzyme	leucine
Enzyme	Lead poisoning	d-aminolevulinate dehydrogenase	lead
Enzyme	Lesch-Nyhan syndrome	Uricase	Uric acid
Enzyme	Maple syrup urine disease	Leucine metabolizing enzyme	Leucine
Enzyme	Methylmalonic acidemia (vitamin b12 non-responsive)	methylmalonyl-CoA mutase	methylmalonate
Enzyme	Mitochondrial neurogastrointestinal encephalomyopathy	thymidine phosphorylase	thymidine
Enzyme	Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE)	Thymidine phosphorylase	Thymidine
Enzyme	Owren's disease	FV	enzyme replacement
Enzyme	p53-null solid tumor	Serine dehyrdatase or serine hydroxymethyl transferase	serine
Enzyme	Pancreatic adenocarcinoma	Asparaginase	asparagine

Enzyme	Phenylketonuria	Phenylalanine hydroxylase, phenylalanine ammonia lyase	Phenylalanine
Enzyme	Primary hyperoxaluria	Oxalate oxidase	Oxalate
Enzyme	Propionic acidemia	Propionate conversion enzyme?	Proprionyl coA
Enzyme	Purine nucleoside phosphorylase deficiency	Purine nucleoside phosphorylase	Inosine, dGTP
Enzyme	Stuart-Power Def.	FX	enzyme replacement
Enzyme	Thrombotic Thrombocytopenic Purpura	ADAMTS13	ultra-large von willebrand factor (ULVWF)
Enzyme	Transferase deficient galactosemia (Galactosemia type 1)	galactose dehydrogenase	Galactose-1-phosphate
Enzyme	Tyrosinemia type 1	tyrosine phenol-lyase	tyrosine
Enzyme	von Willebrand disease	vWF	enzyme replacement
IC clearance	IgA Nephropathy	Complement receptor 1	Immune complexes
IC clearance	Lupus nephritis	Complement receptor 1	immune complex
IC clearance	Systemic lupus erythematosus	Complement receptor 1	immune complex
Infectious	Anthrax (B. anthracis) infection	an antibody-like binder to B. anthracis surface protein	B. anthracis
Infectious	C. botulinum infection	an antibody-like binder to C. botulinum surface protein	C. botulinum
Infectious	C. difficile infection	an antibody-like binder to C. difficile surface protein	C. difficile
Infectious	Candida infection	an antibody-like binder to candida surface protein	candida
Infectious	E. coli infection	an antibody-like binder to E.coli surface protein	E. coli
Infectious	Ebola infection	an antibody-like binder to Ebola surface protein	Ebola
Infectious	Hepatitis B (HBV) infection	an antibody-like binder to HBV surface protein	HBV
Infectious	Hepatitis C (HCV) infection	an antibody-like binder to HCV surface protein	HCV
Infectious	Human immunodeficiency virus (HIV) infection	an antibody-like binder to HIV envelope proteins or CD4 or CCR5 or	HIV
Infectious	M. tuberculosis infection	an antibody-like binder to M. tuberculosis surface protein	M. tuberculosis
Infectious	Malaria (P. falciparum) infection	an antibody-like binder to P. falciparum surface protein	P. falciparum
Lipid	Hepatic lipase deficiency, hypercholesterolemia	Hepatic lipase (LIPC)	Lipoprotein, intermediate density (IDL)
Lipid	Hyperalphalipoproteinemia 1	Cholesteryl ester transfer protein(CETP)	Lipoprotein, high density (HDL)
Lipid	hypercholesterolemia	an antibody-like binder to low-	LDL

		density lipoprotein (LDL), LDL receptor	
Lipid	hypercholesterolemia	an antibody-like binder to high-density lipoprotein (HDL) or HDL receptor	HDL
Lipid	lipoprotein lipase deficiency	lipoprotein lipase	chilomicrons and very low density lipoproteins (VLDL)
Lipid	Lipoprotein lipase deficiency, disorders of lipoprotein metabolism	lipoprotein lipase (LPL)	Lipoprotein, very low density (VLDL)
Lysosomal storage	Aspartylglucosaminuria (208400)	N-Aspartylglucosaminidase	glycoproteins
Lysosomal storage	Cerebrotendinous xanthomatosis (cholestanol lipidosis; 213700)	Sterol 27-hydroxylase	lipids, cholesterol, and bile acid
Lysosomal storage	Ceroid lipofuscinosis Adult form (CLN4, Kufs' disease; 204300)	Palmitoyl-protein thioesterase-1	lipopigments
Lysosomal storage	Ceroid lipofuscinosis Infantile form (CLN1, Santavuori-Haltia disease; 256730)	Palmitoyl-protein thioesterase-1	lipopigments
Lysosomal storage	Ceroid lipofuscinosis Juvenile form (CLN3, Batten disease, Vogt-Spielmeyer disease; 204200)	Lysosomal transmembrane CLN3 protein	lipopigments
Lysosomal storage	Ceroid lipofuscinosis Late infantile form (CLN2, Jansky-Bielschowsky disease; 204500)	Lysosomal pepstatin-insensitive peptidase	lipopigments
Lysosomal storage	Ceroid lipofuscinosis Progressive epilepsy with intellectual disability (600143)	Transmembrane CLN8 protein	lipopigments
Lysosomal storage	Ceroid lipofuscinosis Variant late infantile form (CLN6; 601780)	Transmembrane CLN6 protein	lipopigments
Lysosomal storage	Ceroid lipofuscinosis Variant late infantile form, Finnish type (CLN5; 256731)	Lysosomal transmembrane CLN5 protein	lipopigments
Lysosomal storage	Cholesteryl ester storage disease (CESD)	lisosomal acid lipase	lipids and cholesterol
Lysosomal storage	Congenital disorders of N-glycosylation CDG Ia (solely neurologic and neurologic-multivisceral	Phosphomannomutase-2	N-glycosylated protein

	forms; 212065)		
Lysosomal storage	Congenital disorders of N-glycosylation CDG Ib (602579)	Mannose (Man) phosphate (P) isomerase	N-glycosylated protein
Lysosomal storage	Congenital disorders of N-glycosylation CDG Ic (603147)	Dolicho-P-Glc:Man9GlcNAc2-PP-dolichol glucosyltransferase	N-glycosylated protein
Lysosomal storage	Congenital disorders of N-glycosylation CDG Id (601110)	Dolicho-P-Man:Man5GlcNAc2-PP-dolichol mannosyltransferase	N-glycosylated protein
Lysosomal storage	Congenital disorders of N-glycosylation CDG Ie (608799)	Dolichol-P-mannose synthase	N-glycosylated protein
Lysosomal storage	Congenital disorders of N-glycosylation CDG If (609180)	Protein involved in mannose-P-dolichol utilization	N-glycosylated protein
Lysosomal storage	Congenital disorders of N-glycosylation CDG Ig (607143)	Dolichyl-P-mannose:Man-7-GlcNAc-2-PP-dolichyl-α-6-mannosyltransferase	N-glycosylated protein
Lysosomal storage	Congenital disorders of N-glycosylation CDG Ih (608104)	Dolichyl-P-glucose:Glc-1-Man-9-GlcNAc-2-PP-dolichyl-α-3-glucosyltransferase	N-glycosylated protein
Lysosomal storage	Congenital disorders of N-glycosylation CDG Ii (607906)	α-1,3-Mannosyltransferase	N-glycosylated protein
Lysosomal storage	Congenital disorders of N-glycosylation CDG IIa (212066)	Mannosyl-α-1,6-glycoprotein-β-1,2-N-acetylglucosminyltransferase	N-glycosylated protein
Lysosomal storage	Congenital disorders of N-glycosylation CDG IIb (606056)	Glucosidase I	N-glycosylated protein
Lysosomal storage	Congenital disorders of N-glycosylation CDG IIc (Rambam-Hasharon syndrome; 266265	GDP-fucose transporter-1	N-glycosylated protein
Lysosomal storage	Congenital disorders of N-glycosylation CDG IId (607091)	β-1,4-Galactosyltransferase	N-glycosylated protein
Lysosomal storage	Congenital disorders of N-glycosylation CDG IIe (608779)	Oligomeric Golgi complex-7	N-glycosylated protein
Lysosomal storage	Congenital disorders of N-glycosylation CDG Ij (608093)	UDP-GlcNAc:dolichyl-P NAcGlc phosphotransferase	N-glycosylated protein
Lysosomal storage	Congenital disorders of N-glycosylation CDG Ik (608540)	β-1,4-Mannosyltransferase	N-glycosylated protein
Lysosomal storage	Congenital disorders of N-glycosylation CDG Il (608776)	α-1,2-Mannosyltransferase	N-glycosylated protein

Lysosomal storage	Congenital disorders of N-glycosylation, type I (pre-Golgi glycosylation defects)	α-1,2-Mannosyltransferase	N-glycosylated protein
Lysosomal storage	Cystinosis	Cystinosin (lysosomal cystine transporter)	Cysteine
Lysosomal storage	Fabry's disease (301500)	Trihexosylceramide α-galactosidase	globotriaosylceramide
Lysosomal storage	Farber's disease (lipogranulomatosis; 228000)	Ceramidase	lipids
Lysosomal storage	Fucosidosis (230000)	α-L-Fucosidase	fucose and complex sugars
Lysosomal storage	Galactosialidosis (Goldberg's syndrome, combined neuraminidase and β-galactosidase deficiency; 256540)	Protective protein/cathepsin A (PPCA)	lysosomal content
Lysosomal storage	Gaucher's disease	Glucosylceramide β-glucosidase	sphingolipids
Lysosomal storage	Glutamyl ribose-5-phosphate storage disease (305920)	ADP-ribose protein hydrolase	glutamyl ribose 5-phosphate
Lysosomal storage	Glycogen storage disease type 2 (Pompe's disease)	alpha glucosidase	glycogen
Lysosomal storage	GM1 gangliosidosis, generalized	Ganglioside β-galactosidase	acidic lipid material, gangliosides
Lysosomal storage	GM2 activator protein deficiency (Tay-Sachs disease AB variant, GM2A; 272750)	GM2 activator protein	gangliosides
Lysosomal storage	GM2 gangliosidosis	Ganglioside β-galactosidase	gangliosides
Lysosomal storage	Infantile sialic acid storage disorder (269920)	Na phosphate cotransporter, sialin	sialic acid
Lysosomal storage	Krabbe's disease (245200)	Galactosylceramide β-galactosidase	sphingolipids
Lysosomal storage	Lysosomal acid lipase deficiency (278000)	Lysosomal acid lipase	cholesteryl esters and triglycerides
Lysosomal storage	Metachromatic leukodystrophy (250100)	Arylsulfatase A	sulfatides
Lysosomal storage	Mucolipidosis ML II (I-cell disease; 252500)	N-Acetylglucosaminyl-1-phosphotransfeerase catalytic subunit	N-linked glycoproteins
Lysosomal storage	Mucolipidosis ML III (pseudo-Hurler's polydystrophy)	N-acetylglucosaminyl-1-phosphotransfeerase	N-linked glycoproteins
Lysosomal storage	Mucolipidosis ML III (pseudo-Hurler's polydystrophy) Type III-	Catalytic subunit	N-linked glycoproteins

	A (252600)		
Lysosomal storage	Mucolipidosis ML III (pseudo-Hurler's polydystrophy) Type III-C (252605)	Substrate-recognition subunit	N-linked glycoproteins
Lysosomal storage	Mucopolysaccharidosis MPS I H/S (Hurler-Scheie syndrome; 607015)	α-l-Iduronidase	glycosaminoglycans
Lysosomal storage	Mucopolysaccharidosis MPS I-H (Hurler's syndrome; 607014)	α-l-Iduronidase	glycosaminoglycans
Lysosomal storage	Mucopolysaccharidosis MPS II (Hunter's syndrome; 309900)	Iduronate sulfate sulfatase	glycosaminoglycans
Lysosomal storage	Mucopolysaccharidosis MPS III (Sanfilippo's syndrome) Type III-A (252900)	Heparan-S-sulfate sulfamidase	glycosaminoglycans
Lysosomal storage	Mucopolysaccharidosis MPS III (Sanfilippo's syndrome) Type III-B (252920)	N-acetyl-D-glucosaminidase	glycosaminoglycans
Lysosomal storage	Mucopolysaccharidosis MPS III (Sanfilippo's syndrome) Type III-C (252930)	Acetyl-CoA-glucosaminide N-acetyltransferase	glycosaminoglycans
Lysosomal storage	Mucopolysaccharidosis MPS III (Sanfilippo's syndrome) Type III-D (252940)	N-acetyl-glucosaminine-6-sulfate sulfatase	glycosaminoglycans
Lysosomal storage	Mucopolysaccharidosis MPS I-S (Scheie's syndrome; 607016)	α-l-Iduronidase	glycosaminoglycans
Lysosomal storage	Mucopolysaccharidosis MPS IV (Morquio's syndrome) Type IV-A (253000)	Galactosamine-6-sulfate sulfatase	glycosaminoglycans
Lysosomal storage	Mucopolysaccharidosis MPS IV (Morquio's syndrome) Type IV-B (253010)	β-Galactosidase	glycosaminoglycans
Lysosomal storage	Mucopolysaccharidosis MPS IX (hyaluronidase deficiency; 601492)	Hyaluronidase deficiency	glycosaminoglycans
Lysosomal storage	Mucopolysaccharidosis MPS VI (Maroteaux-Lamy syndrome; 253200)	N-Acetyl galactosamine α-4-sulfate sulfatase (arylsulfatase B)	glycosaminoglycans
Lysosomal storage	Mucopolysaccharidosis MPS VII (Sly's syndrome; 253220)	β-Glucuronidase	glycosaminoglycans

Lysosomal storage	Mucosulfatidosis (multiple sulfatase deficiency; 272200)	Sulfatase-modifying factor-1	sulfatides
Lysosomal storage	Niemann-Pick disease type A	Sphingomyelinase	sphingomyelin
Lysosomal storage	Niemann-Pick disease type B	Sphingomyelinase	sphingomyelin
Lysosomal storage	Niemann-Pick disease Type C1/Type D ((257220)	NPC1 protein	sphingomyelin
Lysosomal storage	Niemann-Pick disease Type C2 (607625)	Epididymal secretory protein 1 (HE1; NPC2 protein)	sphingomyelin
Lysosomal storage	Prosaposin deficiency (176801)	Prosaposin	sphingolipids
Lysosomal storage	Pycnodysostosis (265800)	Cathepsin K	kinins
Lysosomal storage	Sandhoff's disease; 268800	β-Hexosaminidase B	gangliosides
Lysosomal storage	Saposin B deficiency (sulfatide activator deficiency)	Saposin B	sphingolipids
Lysosomal storage	Saposin C deficiency (Gaucher's activator deficiency)	Saposin C	sphingolipids
Lysosomal storage	Schindler's disease Type I (infantile severe form; 609241)	N-Acetyl-galactosaminidase	glycoproteins
Lysosomal storage	Schindler's disease Type II (Kanzaki disease, adult-onset form; 609242)	N-Acetyl-galactosaminidase	glycoproteins
Lysosomal storage	Schindler's disease Type III (intermediate form; 609241)	N-Acetyl-galactosaminidase	glycoproteins
Lysosomal storage	Sialidosis (256550)	Neuraminidase 1 (sialidase)	mucopolysaccharides and mucolipids
Lysosomal storage	Sialuria Finnish type (Salla disease; 604369)	Na phosphate cotransporter, sialin	sialic acid
Lysosomal storage	Sialuria French type (269921)	UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase, sialin	sialic acid
Lysosomal storage	Sphingolipidosis Type I (230500)	Ganglioside β-galactosidase	sphingolipids
Lysosomal storage	Sphingolipidosis Type II (juvenile type; 230600)	Ganglioside β-galactosidase	sphingolipids
Lysosomal storage	Sphingolipidosis Type III (adult type; 230650)	Ganglioside β-galactosidase	sphingolipids
Lysosomal storage	Tay-Sachs disease; 272800	β-Hexosaminidase A	gangliosides
Lysosomal storage	Winchester syndrome (277950)	Metalloproteinase-2	mucopolysaccharides

Lysosomal storage	Wolman's disease	lysosomal acid lipase	lipids and cholesterol
Lysosomal storage	α-Mannosidosis (248500), type I (severe) or II (mild)	α-D-Mannosidase	carbohydrates and glycoproteins
Lysosomal storage	β-Mannosidosis (248510)	β-D-Mannosidase	carbohydrates and glycoproteins
Toxic Molecule	alpha hemolysin poisoning	an antibody-like binder to alpha hemolysin	alpha hemolysin
Toxic Molecule	antrax toxin poisoning	an antibody-like binder to anthrax toxin	anthrax toxin
Toxic Molecule	bacterial toxin-induced shock	an antibody-like binder to bacterial toxin	bacterial toxin
Toxic Molecule	botulinum toxin poisoning	an antibody-like binder to botulinum toxin	botulinum toxin
Toxic Molecule	Hemochromatosis (iron poisoning)	iron chelator	molecular iron
Toxic Molecule	Methanol poisoning	Methanol dehdrogenase	Methanol
Toxic Molecule	Nerve gas poisoning	Butyryl cholinesterase	Sarin
Toxic Molecule	Prion disease caused by PRP	an antibody-like binder to prion protein PRP	Prion protein PRP
Toxic Molecule	Prion disease caused by PRPc	an antibody-like binder to prion protein PRPc	Prion protein PRPc
Toxic Molecule	Prion disease caused by PRPsc	an antibody-like binder to prion protein PRPsc	Prion protein PRPsc
Toxic Molecule	Prion disease caused by PRPres	an antibody-like binder to prion protein PRPres	Prion protein PRPres
Toxic Molecule	Sepsis or cytokine storm	an antibody-like binder to cytokines or Duffy antigen receptor of chemokines (DARC)	cytokines
Toxic Molecule	spider venom poisoning	an antibody-like binder to spider venom	spider venom
Toxic Molecule	Wilson disease	copper chelator	molecular copper

Table 5: Electroporation Conditions (Day 8-9)

Sample	Pulse Voltage	Pulse width	Pulse number	% GFP	Cell viability
1	No electroporation			0.21	97.39
2	1400	20	1	86.9	92.6
3	1500	20	1	79.5	85.7
4	1600	20	1	68.2	78.5
5	1700	20	1	41.4	52.3

6	1100	30	1	79	96
7	1200	30	1	83.6	91.9
8	1300	30	1	77.6	86.6
9	1400	30	1	30.9	42.7
10	1000	40	1	65.3	92.4
11	1100	40	1	69.3	86.9
12	1200	40	1	65.8	79.9
13	1100	20	2	81.3	92.8
14	1200	20	2	82.1	91.2
15	1300	20	2	78.2	86.3
16	1400	20	2	79.3	88.1
17	850	30	2	32.4	95.9
18	950	30	2	59.5	93.8
19	1050	30	2	72	90.8
20	1150	30	2	74.8	84.8
21	1300	10	3	88.3	94.2
22	1400	10	3	88.7	93.3
23	1500	10	3	86.5	90.3
24	1600	10	3	83.3	87.7

Table 6: Electroporation Conditions (Day 12-13)

Sample	Pulse Voltage	Pulse width	Pulse number	% GFP	Cell viability
1	No electroporation			0.58	96.7
2	1400	20	1	42.5	94.9
3	1500	20	1	54.8	91.8
4	1600	20	1	56.9	91.6
5	1700	20	1	61.5	88.7
6	1100	30	1	13.5	95.6
7	1200	30	1	29	95.5

8	1300	30	1	43.8	94.3
9	1400	30	1	44.5	92.9
10	1000	40	1	6.5	95.3
11	1100	40	1	21.7	94.8
12	1200	40	1	33.2	92.3
13	1100	20	2	18	95.8
14	1200	20	2	29.3	95.2
15	1300	20	2	42	94.5
16	1400	20	2	51.5	91.8
17	850	30	2	2.7	95.9
18	950	30	2	7.3	95.3
19	1050	30	2	13.5	94.5
20	1150	30	2	20.7	94.7
21	1300	10	3	27.3	95.9
22	1400	10	3	38.8	95.3
23	1500	10	3	55	94.1
24	1600	10	3	62.6	93.3

Table 7: Electroporation Conditions (Day 14-16)

Sample	Pulse Voltage	Pulse width	Pulse number	% GFP	Cell viability
0	No electroporation			1.1	5.2
1	1700	20	1	44.7	7.7
2	1700	20	2	44.1	15.5
3	1700	20	3	42.7	25
4	1600	10	3	37.6	7.6
5	1600	10	6	34.9	19.1
6	1600	10	8	20.1	47.8
7	1600	20	1	36.7	5.7
8	1600	20	2	37.2	14.6

9	1600	20	3	40.2	13
10	1700	10	1	21.7	4.9
11	1700	10	2	43	9.7
12	1700	10	3	24.9	33.9

Table 8: GFP fluorescence of electroporated Day 4 cells.

	% P1	% GFP+ cells	MFI	% AAD-
Non-electroporated control	87.3	0.85	2,678	98.6
Electroporated, condition A, trial 1	79	91.6	121,279	98.6
Electroporated, condition A, trial 2	80.8	90.6	105,741	98.5
Electroporated, condition B, trial 1	83.5	58.8	25,482	98.4
Electroporated, condition B, trial 2	85.9	19.6	10,709	98.7
Electroporated, condition C, trial 1	87	35	17,086	98.7
Electroporated, condition C, trial 2	86.3	13.1	8,114	98.8

Table 9: GFP fluorescence of Day 4 cells electroporated with chemically modified RNA

	% P1	% GFP+ cells	MFI	% AAD-
Non-electroporated control	87.3	0.85	2,678	98.6
Electroporated, condition A, trial 1	87.2	96.6	75,393	98.0
Electroporated, condition A, trial 2	87.4	96.3	75,853	98.5
Electroporated, condition B, trial 1	88.4	60.8	23,097	98.9
Electroporated, condition B, trial 2	87.6	57.8	21,759	98.7
Electroporated, condition C, trial 1	88.7	61	24,857	98.8
Electroporated, condition C, trial 2	88.4	50.9	20,358	98.5

Table 10: GFP fluorescence of Day 12 cells electroporated with chemically modified RNA

	% P1	% GFP+ cells	MFI	% AAD-
Non-electroporated control	92.2	0.86	3,754	95
Electroporated, trial 1	93.7	55.2	22,748	98
Electroporated, trial 2	90.7	90	107,091	94

Table 11: Evaluation of cell viability and proliferation ability by trypan blue staining after electroporation

	Day 8 Total cells (M)	Day 9 Total Cells (M)	Day 9 Live Cells (M)	Day 9 Cell viability
Electroporated without exogenous nucleic acid	0.21	0.441	0.441	100
Electroporated with unmodified GFP mRNA, 1 ug	0.2	0.376	0.37	99
Electroporated with unmodified GFP mRNA, 2 ug	0.2	0.354	0.332	94
Electroporated with modified GFP mRNA, 1 ug	0.2	0.414	0.381	92

Table 12: human noncoding RNAs

A1BG-AS1 A2M-AS1 A2ML1-AS1 AADACL2-AS1 AATK-AS1 ABCA9-AS1 ABCC5-AS1 ABHD11-AS1 ABHD14A-ACY1 ABHD15-AS1 ACAP2-IT1 ACTA2-AS1 ACTN1-AS1 ACVR2B-AS1 LOC100130964 ADAMTS19-AS1 ADAMTS9-AS1 ADAMTS9-AS2 ADAMTSL4-AS1 ADARB2-AS1 ADD3-AS1 ADGRA1-AS1 ADGRL3-AS1 ADIPOQ-AS1 ADIRF-AS1 ADNP-AS1 ADORA2A-AS1 ADPGK-AS1 LOC104968398 AFAP1-AS1 AFF2-IT1 AGAP1-IT1 AGAP2-AS1 AGBL1-AS1 AGBL4-IT1 AGBL5-AS1 AGPAT4-IT1 AKT3-IT1 ALDH1L1-AS1 ALDH1L1-AS2 ALG9-IT1 ALKBH3-AS1 ALMS1-IT1 ALOX12-AS1 APTR AMMECR1-IT1 ANKRD10-IT1 ANKRD33B-AS1 ANKRD34C-AS1 ANKRD44-IT1 ANO1-AS1 ANO1-AS2 ANP32A-IT1 LOC280665 AIRN UCH1LAS AOAH-IT1 AP4B1-AS1 APCDD1L-AS1 APOA1-AS APOBEC3B-AS1 APOC4-APOC2 AATBC ABALON AQP4-AS1 ARAP1-AS1 ARAP1-AS2 ARHGAP19-SLIT1 ARHGAP22-IT1 ARHGAP26-AS1 ARHGAP26-IT1 ARHGAP31-AS1 ARHGAP5-AS1 ARHGEF19-AS1 ARHGEF26-AS1 ARHGEF3-AS1 ARHGEF38-IT1 ARHGEF7-AS1 ARHGEF7-AS2 ARHGEF7-IT1 ARHGEF9-IT1 ARID4B-IT1 ARL2-SNX15 ARMC2-AS1 ARNTL2-AS1 ARPP21-AS1 ARRDC1-AS1 ARRDC3-AS1 ARSD-AS1 ASAP1-IT1 ASAP1-IT2 ASB16-AS1 ASH1L-AS1 ASMTL-AS1 ASTN2-AS1 ATE1-AS1 ATG10-AS1 ATG10-IT1 ATP11A-AS1 ATP13A4-AS1 ATP13A5-AS1 ATP1A1-AS1 ATP1B3-AS1 ATP2A1-AS1 ATP2B2-IT1 ATP2B2-IT2 ATP6V0E2-AS1 ATP6V1B1-AS1 ATP6V1G2-DDX39B ATXN8OS AZIN1-AS1 LOC100506431 B3GALT5-AS1 B4GALT1-AS1 B4GALT4-AS1 BAALC-AS1 BAALC-AS2 BACE1-AS BACE2-IT1 BACH1-IT2 BACH1-IT3 BAIAP2-AS1 BARX1-AS1 BBOX1-AS1 BCDIN3D-AS1 BLACE BDNF-AS BEAN1-AS1 BGLT3 BHLHE40-AS1 BIN3-IT1 BIRC6-AS1 BIRC6-AS2 BLACAT1 BPESC1 BLOC1S1-RDH5 BLOC1S5-TXNDC5 BMP7-AS1 BMPR1B-AS1 BOK-AS1 BOLA3-AS1 BANCR BCYRN1 BRE-AS1 BCAR4 BREA2 BRWD1-AS1 BRWD1-IT2 BSN-AS2 BISPR BTBD9-AS1 BVES-AS1 BZRAP1-AS1 C10orf32-ASMT C10orf71-AS1 C15orf59-AS1 C1QTNF1-AS1 C1QTNF3-AMACR C1QTNF9-AS1 C1RL-AS1 C2-AS1 C20orf166-AS1 C21orf62-AS1 C21orf91-OT1 C3orf67-AS1 C5orf66-AS1 C5orf66-AS2 C8orf34-AS1 C8orf37-AS1 C9orf135-AS1 C9orf173-AS1 C9orf41-AS1 CA3-AS1 CACNA1C-AS1 CACNA1C-AS2 CACNA1C-AS4 CACNA1C-IT1 CACNA1C-IT2 CACNA1C-IT3 CACNA1G-AS1 CACNA2D3-AS1 CACTIN-AS1 CADM2-AS1 CADM2-AS2 CADM3-AS1 CALML3-AS1 CAMTA1-IT1 CASC11 CASC15 CASC16 CASC17 CASC18 CASC19 CASC2 CASC20 CASC21 CASC23 CASC6 CASC8 CASC9 CAPN10-AS1 CARD8-AS1 CARS-AS1 CASK-AS1 CECR3 CECR7 CATIP-AS1 CATIP-AS2 CATR1 CBR3-AS1 CCDC13-AS1 CCDC144NL-AS1 CCDC148-AS1 CCDC183-AS1 CCDC26 CCDC37-AS1 CCDC39-AS1 CCL15-CCL14 CCND2-AS1 CCNT2-AS1 CD27-AS1 CD81-AS1 CDC37L1-AS1 CDC42-IT1 CDH23-AS1 CDIPT-AS1 CDKN1A-AS1 CDKN2A-AS1 CDKN2B-AS1 CDR1-AS CEBPA-AS1 CEBPB-AS1 CEBPZOS CECR5-AS1 CELF2-AS1 CELF2-AS2 CELSR3-AS1 CEP83-AS1 CERS3-AS1 CERS6-AS1 CCHE1 CFAP44-AS1 CFAP58-AS1 CFLAR-AS1 CHKB-AS1 CHKB-CPT1B CHL1-AS1 CHODL-AS1 CISTR CHRM3-AS1 CHRM3-AS2 C1orf145 C1orf220 C11orf39 C11orf72 C14orf144 C18orf15 C3orf49 C5orf17 C5orf56 C6orf7 C7orf13 C8orf49 CIRBP-AS1 CKMT2-AS1 CLDN10-AS1 CLIP1-AS1 CLSTN2-AS1 CLUHP3 CLYBL-AS1 CLYBL-AS2 CDRT7 CDRT8 CNOT10-AS1 CNTFR-AS1 CNTN4-AS1 CNTN4-AS2 COL18A1-AS1 COL18A1-AS2 COL4A2-AS1 COL5A1-AS1 LOC387720 CAHM CCAT1 CCAT2 CRNDE COPG2IT1 COX10-AS1 CPB2-AS1 CPEB1-AS1 CPEB2-AS1 CPS1-IT1 CRHR1-IT1 CRTC3-AS1 CRYM-AS1 CSE1L-AS1 CSMD2-AS1 CSNK1G2-AS1 CSTF3-AS1 CTBP1-AS CTBP1-AS2 CYP17A1-AS1 CYP1B1-AS1 CYP4A22-AS1

CYP51A1-AS1 D21S2088E DBET DAB1-AS1 DACT3-AS1 DAOA-AS1 DAPK1-IT1 DARS-AS1 DBH-AS1 DCTN1-AS1 DCUN1D2-AS DDC-AS1 DDX11-AS1 DDX26B-AS1 LOC642846 DLEU1 DLEU2 DENND5B-AS1 DEPDC1-AS1 DGUOK-AS1 DHRS4-AS1 DIAPH2-AS1 DIAPH3-AS1 DIAPH3-AS2 DICER1-AS1 DANCR DGCR10 DGCR11 DGCR7 DGCR9 DIO2-AS1 DIO3OS DIP2A-IT1 DISC1FP1 DISC1-IT1 DIRC3 DISC2 PKI55 DLEU1-AS1 DLEU7-AS1 DLG1-AS1 DLG3-AS1 DLG5-AS1 DLGAP1-AS1 DLGAP1-AS2 DLGAP1-AS3 DLGAP1-AS4 DLGAP1-AS5 DLGAP2-AS1 DLGAP4-AS1 DLX2-AS1 DLX6-AS1 DLX6-AS2 DMD-AS3 DNAH17-AS1 DNAJB5-AS1 DNAJB8-AS1 DNAJC27-AS1 DNAJC3-AS1 DNAJC9-AS1 DNM3-IT1 DNM3OS DNMBP-AS1 DALIR DOCK4-AS1 DOCK9-AS1 DOCK9-AS2 DSCR10 DSCR8 DSCR9 DRAIC DPH6-AS1 DPP10-AS1 DPP10-AS3 DPYD-AS1 DPYD-AS2 DPYD-IT1 DSCAS DSCAM-AS1 DSCAM-IT1 DSCR4-IT1 DSG1-AS1 DSG2-AS1 DNAH10OS DYX1C1-CCPG1 ERICD E2F3-IT1 EAF1-AS1 EDNRB-AS1 EDRF1-AS1 EEF1E1-BLOC1S5 EFCAB14-AS1 EFCAB6-AS1 EFCAB10 EGFLAM-AS1 EGFLAM-AS2 EGFLAM-AS3 EGFLAM-AS4 EGFR-AS1 ELDR EHD4-AS1 EHHADH-AS1 EHMT1-IT1 EIF1AX-AS1 EIF1B-AS1 EIF2B5-AS1 EIF2B5-IT1 EIF3J-AS1 ELFN1-AS1 ELMO1-AS1 ELOVL2-AS1 ESRG EMC3-AS1 EML2-AS1 EMX2OS LOC105376387 ERVK13-1 ENO1-AS1 ENOX1-AS1 ENOX1-AS2 ENTPD1-AS1 ENTPD3-AS1 EGOT EP300-AS1 EPB41L4A-AS1 EPB41L4A-AS2 EPHA1-AS1 EPHA5-AS1 EPN2-AS1 EPN2-IT1 ERC2-IT1 ERI3-IT1 ERICH1-AS1 ERICH3-AS1 ERICH6-AS1 ETV5-AS1 EVX1-AS EWSAT1 EXOC3-AS1 EXTL3-AS1 EZR-AS1 F10-AS1 F11-AS1 FAM13A-AS1 FAM155A-IT1 FAM167A-AS1 FAM170B-AS1 FAM181A-AS1 FAM212B-AS1 FAM222A-AS1 FAM24B-CUZD1 FAM53B-AS1 FAM83A-AS1 FAM83C-AS1 FAM83H-AS1 FAM106A FAM106B FAM106CP FAM138A FAM138B FAM138C FAM138D FAM138E FAM138F FAM157C FAM182A FAM182B FAM183CP FAM197Y4 FAM197Y5 FAM197Y7 FAM201A FAM215A FAM223A FAM223B FAM224A FAM224B FAM225A FAM225B FAM226A FAM226B FAM230B FAM230C FAM231D FAM27B FAM27C FAM27D1 FAM27E2 FAM27E3 FAM27L FAM41AY1 FAM41AY2 FAM41C FAM66A FAM66B FAM66C FAM66D FAM66E FAM74A1 FAM74A3 FAM74A4 FAM74A6 FAM74A7 FAM85A FAM87A FAM87B FAM95A FAM95B1 FAM95C FAM99A FAM99B FANK1-AS1 FARP1-AS1 FAS-AS1 FAR2P1 FAR2P2 FAR2P3 FBXL19-AS1 FBXO22-AS1 FBXO3-AS1 FBXO36-IT1 FER1L6-AS1 FER1L6-AS2 FEZF1-AS1 FGD5-AS1 FGF10-AS1 FGF12-AS1 FGF12-AS2 FGF12-AS3 FGF13-AS1 FGF14-AS1 FGF14-AS2 FGF14-IT1 FIRRE FKBP1A-SDCBP2 FKSG29 FLG-AS1 FLJ16171 FLJ26850 FLJ33360 FLJ35934 FLJ43879 FLJ45079 FLNB-AS1 FLVCR1-AS1 FMR1-AS1 FNDC1-IT1 FOCAD-AS1 FALEC FTCDNL1 FOXCUT FOXC2-AS1 FOXD2-AS1 FOXD3-AS1 FENDRR FOXG1-AS1 FOXN3-AS1 FOXN3-AS2 FOXP1-AS1 FOXP4-AS1 FREM2-AS1 FRMD6-AS1 FRMD6-AS2 FRMPD3-AS1 FRMPD4-AS1 FRY-AS1 FSIP2-AS1 FTCD-AS1 FTO-IT1 FTX FUT8-AS1 FZD10-AS1 GABPB1-AS1 GABRG3-AS1 GAS5-AS1 GAS6-AS1 GAS6-AS2 GAS8-AS1 GAPLINC GACAT1 GACAT2 GACAT3 GCRG224 GHET1 GATA2-AS1 GATA3-AS1 GATA6-AS1 GCSAML-AS1 GAEC1 GFOD1-AS1 GHRLOS GJA9-MYCBP GK-AS1 GLIDR GLIS2-AS1 GLIS3-AS1 GLYCTK-AS1 GMDS-AS1 GNA14-AS1 GNAS-AS1 GNG12-AS1 GPC5-AS1 GPC5-AS2 GPC5-IT1 GPC6-AS1 GPC6-AS2 GPR1-AS GPR158-AS1 GRID1-AS1 GRIK1-AS1 GRIK1-AS2 GRK5-IT1 GRM5-AS1 GRM7-AS1 GRM7-AS2 GRM7-AS3 GAS5 GDF5OS GRTP1-AS1 GSN-AS1 GTF3C2-AS1 GTSE1-AS1 GYG2-AS1 H19 H1FX-AS1 HAGLROS HAND2-AS1 HAO2-IT1 HAS2-AS1 HCFC1-AS1 LOC100131635 LOC646268 LOC728040 LOC100130298 LOC729970 LOC727925 HDAC11-AS1 HECTD2-AS1 HPYR1 HELLPAR HCCAT5 HEIH HULC HEXA-AS1 HEXDC-IT1 HHATL-AS1 HHIP-AS1 HID1-AS1 HIF1A-AS1 HIF1A-AS2 HAR1A HAR1B HIPK1-AS1 HCG11 HCG14 HCG17 HCG18 HCG20 HCG21 HCG22 HCG23 HCG24 HCG25 HCG26 HCG27 HCG4 HCG4B HCG8 HCG9 HCP5 HLA-F-AS1 HLCS-IT1 HLTF-AS1 HLX-AS1 HM13-AS1 HMGN3-AS1 HMMR-AS1 HNF1A-AS1 HNF4A-AS1 HNRNPU-AS1 HNRNPUL2-BSCL2 HORMAD2-AS1 HOTAIR HOXA-AS2 HOXA-AS3 HOTTIP HOTAIRM1 HOXA10-AS HOXA10-HOXA9 HOXA11-AS HOXB-AS1 HOXB-AS2 HOXB-AS3 HOXB-AS4 HOXC-AS1 HOXC-AS2 HOXC-AS3 HOXC13-AS HAGLR HOXD-AS2 HPN-AS1 HS1BP3-IT1 HS6ST2-AS1 HSPB2-C11orf52 HTR2A-AS1 HTR5A-AS1 HTT-AS HPVC1 HYMAI HYI-AS1 IBA57-AS1 ID2-AS1 IDH1-AS1 IDI2-AS1 IFNG-AS1 IFT74-AS1 IGBP1-AS1 IRAIN IGF2-AS IGF2BP2-AS1 IGFBP7-AS1 IGSF11-AS1 IL10RB-AS1 IL12A-AS1 IL21-AS1 IL21R-AS1 ILF3-AS1 IPW INE1 INE2 INHBA-AS1 INMT-FAM188B INO80B-WBP1 INTS6-AS1 IPO11-LRRC70 IPO9-AS1 IQCF5-AS1 IQCH-AS1 IQCJ-SCHIP1-AS1 ISM1-AS1 ISPD-AS1 ISX-AS1 ITCH-IT1 ITFG1-AS1 ITGA9-AS1 ITGB2-AS1 ITGB5-AS1 ITIH4-AS1 ITPK1-AS1 ITPKB-AS1 ITPKB-IT1 ITPR1-AS1 JADRR JAKMIP2-AS1 JARID2-AS1 JAZF1-AS1 JHDM1D-AS1 JMJD1C-AS1 JPX JRKL-AS1 KANSL1-AS1 KBTBD11-OT1 KCNAB1-AS1 KCNAB1-AS2 KCNC4-AS1 KCND3-AS1 KCND3-IT1 KCNH1-IT1 KCNIP2-AS1 KCNIP4-IT1 KCNJ2-AS1 KCNJ6-AS1 KCNMA1-AS1 KCNMA1-AS2 KCNMA1-AS3 KCNMB2-AS1 KCNQ1-AS1 KCNQ1DN KCNQ1OT1 KCNQ5-AS1 KCNQ5-IT1 KCTD21-AS1 KDM4A-AS1 KANTR KDM5C-IT1 KC6 KIAA0087 KIAA0125 KIAA1656 KIAA1875 KIF25-AS1 KIF9-AS1 KIRREL3-AS1 KIRREL3-AS2 KIRREL3-AS3 KIZ-AS1 KLF3-AS1 KLF7-IT1 KLHL30-AS1 KLHL6-AS1 KLHL7-AS1 KMT2E-AS1 KRBOX1-AS1 KRT73-AS1 KRTAP5-AS1 KTN1-AS1 L3MBTL4-AS1 LACTB2-AS1

LAMA5-AS1 LAMP5-AS1 LAMTOR5-AS1 LANCL1-AS1 LARGE-AS1 LARGE-IT1 LARS2-AS1 LATS2-AS1 LBX1-AS1 LBX2-AS1 LCMT1-AS1 LCMT1-AS2 LDLRAD4-AS1 LEF1-AS1 LEMD1-AS1 LENG8-AS1 LRRC37A5P LUNAR1 LGALS8-AS1 LHFPL3-AS1 LHFPL3-AS2 LHX4-AS1 LHX5-AS1 LIFR-AS1 LIMD1-AS1 LIMS3-LOC440895 LINGO1-AS1 LINGO1-AS2 LIPE-AS1 LLPH-AS1 LMCD1-AS1 LMF1-AS1 LMLN-AS1 LMO7-AS1 LMO7DN-IT1 LNX1-AS1 LNX1-AS2 LOC100630923 LOC100499484-C9ORF174 LINC01000 LINC01001 LINC01002 LINC01003 LINC01004 LINC01005 LINC01006 LINC01007 LINC01010 LINC01011 LINC01012 LINC01013 LINC01014 LINC01015 LINC01016 LINC01017 LINC01018 LINC01019 LINC00102 LINC01020 LINC01021 LINC01023 LINC01024 LINC01028 LINC01029 LINC01030 LINC01031 LINC01032 LINC01033 LINC01036 LINC01038 LINC01039 LINC01040 LINC01043 LINC01044 LINC01046 LINC01047 LINC01048 LINC01049 LINC01050 LINC01053 LINC01054 LINC01055 LINC01056 LINC01057 LINC01058 LINC01059 LINC00106 LINC01060 LINC01061 LINC01063 LINC01065 LINC01068 LINC01069 LINC01070 LINC01072 LINC01075 LINC01080 LINC01081 LINC01082 LINC01085 LINC01087 LINC01088 LINC01089 LINC01090 LINC01091 LINC01093 LINC01094 LINC01095 LINC01096 LINC01097 LINC01098 LINC01099 LINC01100 LINC01101 LINC01102 LINC01103 LINC01104 LINC01105 LINC01106 LINC01107 LINC01108 LINC01109 LINC00111 LINC01111 LINC01114 LINC01115 LINC01116 LINC01117 LINC01118 LINC01119 LINC00112 LINC01120 LINC01121 LINC01122 LINC01123 LINC01124 LINC01125 LINC01126 LINC01127 LINC01128 LINC00113 LINC01132 LINC01133 LINC01134 LINC01135 LINC01136 LINC01137 LINC01138 LINC01139 LINC00114 LINC01140 LINC01141 LINC01142 LINC01143 LINC01144 LINC01146 LINC01149 LINC00115 LINC01150 LINC01151 LINC01152 LINC01153 LINC01158 LINC01159 LINC00116 LINC01160 LINC01162 LINC01163 LINC01164 LINC01166 LINC01167 LINC01168 LINC01169 LINC01170 LINC01176 LINC01177 LINC01179 LINC01180 LINC01182 LINC01184 LINC01185 LINC01186 LINC01187 LINC01189 LINC01191 LINC01192 LINC01193 LINC01194 LINC01195 LINC01197 LINC01198 LINC01201 LINC01202 LINC01203 LINC01204 LINC01205 LINC01206 LINC01207 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LINC00235 LINC00237 LINC00238 LINC00239 LINC00240 LINC00242 LINC00243 LINC00244 LINC00251
LINC00254 LINC00260 LINC00261 LINC00264 LINC00265 LINC00266-1 LINC00266-3 LINC00268
LINC00269 LINC00271 LINC00272 LINC00273 LINC00276 LINC00278 LINC00279 LINC00028 LINC00280
LINC00282 LINC00283 LINC00284 LINC00029 LINC00290 LINC00293 LINC00294 LINC00297 LINC00298
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MIR512-2 MIR513A1 MIR513A2 MIR513B MIR513C MIR514A1 MIR514A2 MIR514A3 MIR514B MIR515-1 MIR515-2 MIR516A1 MIR516A2 MIR516B1 MIR516B2 MIR517A MIR517B MIR517C MIR5186 MIR5187 MIR5188 MIR5189 MIR518A1 MIR518A2 MIR518B MIR518C MIR518D MIR518E MIR518F MIR5190 MIR5191 MIR5192 MIR5193 MIR5194 MIR5195 MIR5196 MIR5197 MIR519A1 MIR519A2 MIR519B MIR519C MIR519D MIR519E MIR520A MIR520B MIR520C MIR520D MIR520E MIR520F MIR520G MIR520H MIR521-1 MIR521-2 MIR522 MIR523 MIR524 MIR525 MIR526A1 MIR526A2 MIR526B MIR527 MIR532 MIR539 MIR541 MIR542 MIR543 MIR544A MIR544B MIR545 MIR548A1 MIR548A2 MIR548A3 MIR548AA1 MIR548AA2 MIR548AB MIR548AC MIR548AD MIR548AE1 MIR548AE2 MIR548AG1 MIR548AG2 MIR548AH MIR548AI MIR548AJ1 MIR548AJ2 MIR548AK MIR548AL MIR548AM MIR548AN MIR548AO MIR548AP MIR548AQ MIR548AR MIR548AS MIR548AT MIR548AU MIR548AV MIR548AW MIR548AX MIR548AY MIR548AZ MIR548B MIR548BA MIR548BB MIR548C MIR548D1 MIR548D2 MIR548E MIR548F1 MIR548F2 MIR548F3 MIR548F4 MIR548F5 MIR548G MIR548H1 MIR548H2 MIR548H3 MIR548H4 MIR548H5 MIR548I1 MIR548I2 MIR548I3 MIR548I4 MIR548J MIR548K MIR548L MIR548M MIR548N MIR548O MIR548O2 MIR548P MIR548Q MIR548S MIR548T MIR548U MIR548V MIR548W MIR548X MIR548X2 MIR548Y MIR548Z MIR549A MIR550A1 MIR550A2 MIR550A3 MIR550B1 MIR550B2 MIR551A MIR551B MIR552 MIR553 MIR554 MIR555 MIR556 MIR557 MIR5571 MIR5572 MIR5579 MIR558 MIR5580 MIR5581 MIR5582 MIR5583-1 MIR5583-2 MIR5584 MIR5585 MIR5586 MIR5587 MIR5588 MIR5589 MIR559 MIR5590 MIR5591 MIR561 MIR562 MIR563 MIR564 MIR566 MIR567 MIR568 MIR5680 MIR5681A MIR5681B MIR5682 MIR5683 MIR5684 MIR5685 MIR5687 MIR5688 MIR5689 MIR569 MIR5690 MIR5691 MIR5692A1 MIR5692A2 MIR5692B MIR5692C1 MIR5692C2 MIR5693 MIR5694 MIR5695 MIR5696 MIR5697 MIR5698 MIR5699 MIR570 MIR5700 MIR5701-1 MIR5701-2 MIR5701-3 MIR5702 MIR5703 MIR5704 MIR5705 MIR5706 MIR5707 MIR5708 MIR571 MIR572 MIR573 MIR5739 MIR574 MIR575 MIR576 MIR577 MIR578 MIR5787 MIR579 MIR580 MIR581 MIR582 MIR583 MIR584 MIR585 MIR586 MIR587 MIR588 MIR589 MIR590 MIR591 MIR592 MIR593 MIR595 MIR596 MIR597 MIR598 MIR599 MIR600 MIR601 MIR602 MIR603 MIR604 MIR605 MIR606 MIR6068 MIR6069 MIR607 MIR6070 MIR6071 MIR6072 MIR6073 MIR6074 MIR6075 MIR6076 MIR6077 MIR6078 MIR6079 MIR608 MIR6080 MIR6081 MIR6082 MIR6083 MIR6084 MIR6085 MIR6086 MIR6087 MIR6088 MIR6089 MIR609 MIR6090 MIR610 MIR611 MIR612 MIR6124 MIR6125 MIR6126 MIR6127 MIR6128 MIR6129 MIR613 MIR6130 MIR6131 MIR6132 MIR6133 MIR6134 MIR614 MIR615 MIR616 MIR6165 MIR617 MIR618 MIR619 MIR620 MIR621 MIR622 MIR623 MIR624 MIR625 MIR626 MIR627 MIR628 MIR629 MIR630 MIR631 MIR632 MIR633 MIR634 MIR635 MIR636 MIR637 MIR638 MIR639 MIR640 MIR641 MIR642A MIR642B MIR643 MIR644A MIR645 MIR646 MIR647 MIR648 MIR649 MIR6499 MIR650 MIR6500 MIR6501 MIR6502 MIR6503 MIR6504 MIR6505 MIR6506 MIR6507 MIR6508 MIR6509 MIR651 MIR6510 MIR6511A1 MIR6511A2 MIR6511A3 MIR6511A4 MIR6511B1 MIR6511B2 MIR6512 MIR6513 MIR6514 MIR6515 MIR6516 MIR652 MIR653 MIR654 MIR655 MIR656 MIR657 MIR658 MIR659 MIR660 MIR661 MIR662 MIR663A MIR663B MIR664A MIR664B MIR665 MIR668 MIR670 MIR671 MIR6715A MIR6715B MIR6716 MIR6717 MIR6718 MIR6719 MIR6720 MIR6721 MIR6722 MIR6723 MIR6724-1 MIR6724-2 MIR6724-3 MIR6724-4 MIR6726 MIR6727 MIR6728 MIR6729 MIR6730 MIR6731 MIR6732 MIR6733 MIR6734 MIR6735 MIR6736 MIR6737 MIR6738 MIR6739 MIR6740 MIR6741 MIR6742 MIR6743 MIR6744 MIR6745 MIR6746 MIR6747 MIR6748 MIR6749 MIR675 MIR6750 MIR6751 MIR6752 MIR6753 MIR6754 MIR6755 MIR6756 MIR6757 MIR6758 MIR6759 MIR676 MIR6760 MIR6761 MIR6762 MIR6763 MIR6764 MIR6765 MIR6766 MIR6767 MIR6768 MIR6769A MIR6769B MIR6770-1 MIR6770-2 MIR6770-3 MIR6771 MIR6772 MIR6773 MIR6774 MIR6775 MIR6776 MIR6777 MIR6778 MIR6779 MIR6780A MIR6780B MIR6781 MIR6782 MIR6783 MIR6784 MIR6785 MIR6786 MIR6787 MIR6788 MIR6789 MIR6790 MIR6791 MIR6792 MIR6793 MIR6794 MIR6795 MIR6796 MIR6797 MIR6798 MIR6799 MIR6800 MIR6801 MIR6802 MIR6803 MIR6804 MIR6805 MIR6806 MIR6807 MIR6808 MIR6809 MIR6810 MIR6811 MIR6812 MIR6813 MIR6814 MIR6815 MIR6816 MIR6817 MIR6818 MIR6819 MIR6820 MIR6821 MIR6822 MIR6823 MIR6824 MIR6825 MIR6826 MIR6827 MIR6828 MIR6829 MIR6830 MIR6831 MIR6832 MIR6833 MIR6834 MIR6835 MIR6836 MIR6837 MIR6838 MIR6839 MIR6840 MIR6841 MIR6842 MIR6843 MIR6844 MIR6845 MIR6846 MIR6847 MIR6848 MIR6849 MIR6850 MIR6851 MIR6852 MIR6853 MIR6854 MIR6855 MIR6856 MIR6857 MIR6858 MIR6859-1 MIR6859-2 MIR6859-3 MIR6859-4 MIR6860 MIR6861 MIR6862-1 MIR6862-2 MIR6863 MIR6864 MIR6865 MIR6866 MIR6867 MIR6868 MIR6869 MIR6870 MIR6871 MIR6872 MIR6873 MIR6874 MIR6875 MIR6876 MIR6877 MIR6878 MIR6879 MIR6880 MIR6881 MIR6882 MIR6883 MIR6884 MIR6885 MIR6886 MIR6887 MIR6888 MIR6889 MIR6890 MIR6891 MIR6892 MIR6893 MIR6894 MIR6895 MIR708 MIR7-1 MIR7106 MIR7107 MIR7108 MIR7109 MIR711 MIR7110 MIR7111 MIR7112 MIR7113 MIR7114 MIR7150 MIR7151 MIR7152 MIR7153 MIR7154 MIR7155 MIR7156 MIR7157 MIR7158 MIR7159 MIR7160 MIR7161 MIR7162 MIR718 MIR7-2 MIR7-3

MIR744 MIR7515 MIR758 MIR759 MIR760 MIR761 MIR762 MIR764 MIR7641-1 MIR7641-2 MIR765 MIR766 MIR767 MIR769 MIR770 MIR7702 MIR7703 MIR7704 MIR7705 MIR7706 MIR7843 MIR7844 MIR7845 MIR7846 MIR7847 MIR7848 MIR7849 MIR7850 MIR7851 MIR7852 MIR7853 MIR7854 MIR7855 MIR7856 MIR7973-1 MIR7973-2 MIR7974 MIR7975 MIR7976 MIR7977 MIR7978 MIR802 MIR8052 MIR8053 MIR8054 MIR8055 MIR8056 MIR8057 MIR8058 MIR8059 MIR8060 MIR8061 MIR8062 MIR8063 MIR8064 MIR8065 MIR8066 MIR8067 MIR8068 MIR8069-1 MIR8069-2 MIR8070 MIR8071-1 MIR8071-2 MIR8072 MIR8073 MIR8074 MIR8075 MIR8076 MIR8077 MIR8078 MIR8079 MIR8080 MIR8081 MIR8082 MIR8083 MIR8084 MIR8085 MIR8086 MIR8087 MIR8088 MIR8089 MIR8485 MIR873 MIR874 MIR875 MIR876 MIR877 MIR885 MIR887 MIR888 MIR889 MIR890 MIR891A MIR891B MIR892A MIR892B MIR892C MIR9-1 MIR9-2 MIR920 MIR921 MIR922 MIR924 MIR92A1 MIR92A2 MIR92B MIR93 MIR9-3 MIR933 MIR934 MIR935 MIR936 MIR937 MIR938 MIR939 MIR940 MIR941-1 MIR941-2 MIR941-3 MIR941-4 MIR941-5 MIR942 MIR943 MIR944 MIR95 MIR9500 MIR96 MIR98 MIR99A MIR99B MIRLET7A1 MIRLET7A2 MIRLET7A3 MIRLET7B MIRLET7C MIRLET7D MIRLET7E MIRLET7F1 MIRLET7F2 MIRLET7G MIRLET7I MID1IP1-AS1 MIF-AS1 MIR100HG MIR124-2HG MIR133A1HG MIR137HG MIR143HG MIR155HG MIR17HG MIR181A1HG MIR181A2HG MIR202HG MIR2052HG MIR210HG MIR217HG MIR22HG MIR222HG MIR31HG MIR325HG MIR3663HG MIR381HG MIR3976HG MIR4435-2HG MIR4458HG MIR4500HG MIR4697HG MIR497HG MIR503HG MIR600HG MIR646HG MIR663AHG MIR670HG MIR7-3HG MIR7515HG MIR762HG MIR99AHG MIRLET7BHG MIRLET7DHG MIS18A-AS1 MT-LIPCAR MKLN1-AS MKNK1-AS1 MKRN3-AS1 MKX-AS1 MLIP-AS1 MLIP-IT1 MLK7-AS1 MLLT4-AS1 MME-AS1 MMP24-AS1 MMP25-AS1 MNX1-AS1 MORC1-AS1 MORC2-AS1 MORF4L2-AS1 MPRIP-AS1 MRGPRF-AS1 MRGPRG-AS1 MROH7-TTC4 MRPL23-AS1 MRVI1-AS1 MSC-AS1 MSH5-SAPCD1 MTOR-AS1 MTUS2-AS1 MTUS2-AS2 MYB-AS1 MYCBP2-AS1 MYCBP2-AS2 MYCNOS MYCNUT MDS2 MYLK-AS1 MYLK-AS2 MYO16-AS1 MYO16-AS2 MIAT MYHAS MHRT MYT1L-AS1 MZF1-AS1 N4BP2L2-IT2 NAALADL2-AS1 NAALADL2-AS2 NAALADL2-AS3 NADK2-AS1 NAGPA-AS1 NALCN-AS1 NAPA-AS1 NARF-IT1 NAV2-AS1 NAV2-AS2 NAV2-AS3 NAV2-AS4 NAV2-AS5 NAV2-IT1 NCAM1-AS1 NCBP2-AS1 NCBP2-AS2 NCK1-AS1 NCKAP5-IT1 NCOA7-AS1 LOC104968399 NDFIP2-AS1 NDP-AS1 NDUFA6-AS1 NDUFB2-AS1 NDUFV2-AS1 NEBL-AS1 NRAV NRIR NEGR1-IT1 NBR2 NEURL1-AS1 NBAT1 NHEG1 NEXN-AS1 NFIA-AS1 NFIA-AS2 NKILA NFYC-AS1 NHS-AS1 NIFK-AS1 NIPBL-AS1 NKX2-1-AS1 NKX2-2-AS1 NLGN1-AS1 NLGN4Y-AS1 NNT-AS1 NCRNA00250 NAMA NRON NCRUPAR NOP14-AS1 NPHP3-AS1 NPHP3-ACAD11 NPPA-AS1 NPSR1-AS1 NPTN-IT1 NR2F1-AS1 NR2F2-AS1 NREP-AS1 NRG1-IT1 NRG1-IT3 NRG3-AS1 NRSN2-AS1 NTM-IT NTRK3-AS1 NUCB1-AS1 NEAT1 NUP50-AS1 NUTM2A-AS1 NUTM2B-AS1 OCIAD1-AS1 OGFR-AS1 OIP5-AS1 OLMALINC OPA1-AS1 OPCML-IT1 OPCML-IT2 OR2A1-AS1 OSBPL10-AS1 OSER1-AS1 OSGEPL1-AS1 OSMR-AS1 OTUD6B-AS1 OTX2-AS1 OVAAL OVCH1-AS1 OVOL1-AS1 OXCT1-AS1 P2RX5-TAX1BP3 P3H2-AS1 P4HA2-AS1 LOC104940763 PRG1 PABPC1L2B-AS1 PABPC5-AS1 PACRG-AS1 PAN3-AS1 PTCSC1 PTCSC2 PTCSC3 PAPPA-AS1 PAPPA-AS2 PAQR9-AS1 PARD3-AS1 PARD6G-AS1 PAUPAR PAX8-AS1 PAXBP1-AS1 PAXIP1-AS1 PAXIP1-AS2 PCBP1-AS1 PCBP2-OT1 PCCA-AS1 PCDH9-AS1 PCDH9-AS2 PCDH9-AS3 PCDH9-AS4 PCED1B-AS1 PCGEM1 PCNA-AS1 PCOLCE-AS1 PCYT1B-AS1 PDCD4-AS1 PDX1-AS1 PDZRN3-AS1 PEG3-AS1 PEX5L-AS1 PEX5L-AS2 PGM5-AS1 PGM5P3-AS1 PGM5P4-AS1 PHEX-AS1 PHKA1-AS1 PHKA2-AS1 HPBP PGM5P3 PIK3CD-AS1 PIK3CD-AS2 PIK3IP1-AS1 PINK1-AS PIR-FIGF PITPNA-AS1 PITRM1-AS1 PKIA-AS1 PKN2-AS1 PKNOX2-AS1 PLA2G4E-AS1 PLBD1-AS1 PLCB1-IT1 PLCB2-AS1 PLCE1-AS1 PLCE1-AS2 PLCG1-AS1 PLCH1-AS1 PLCH1-AS2 PLCL2-AS1 PLCXD2-AS1 PLS1-AS1 PLS3-AS1 PLSCR5-AS1 PISRT1 POT1-AS1 POTEH-AS1 POU6F2-AS1 POU6F2-AS2 PPEF1-AS1 PPP1R26-AS1 PPP2R2B-IT1 PPP3CB-AS1 PPP4R1-AS1 PPT2-EGFL8 PWAR1 PWAR4 PWAR5 PWAR6 PWARSN PWRN1 PWRN2 PWRN3 PWRN4 PRC1-AS1 PRH1-PRR4 PRICKLE2-AS1 PRICKLE2-AS2 PRICKLE2-AS3 PRNT PRKAG2-AS1 PRKAR2A-AS1 PRKCA-AS1 PRKCQ-AS1 PRKG1-AS1 PRKX-AS1 PRMT5-AS1 LOC101054525 PRR26 PANDAR PARTICL PROSER2-AS1 PART1 PCA3 PRNCR1 PCAT1 PCAT14 PCAT18 PCAT19 PCAT2 PCAT29 PCAT4 PCAT6 PCAT7 LOC440313 PROX1-AS1 PRR34-AS1 PRR7-AS1 PRRT3-AS1 PRRX2-AS1 PSMA3-AS1 PSMB8-AS1 PSMD5-AS1 PSMD6-AS2 PSMG3-AS1 PRINS PSORS1C3 PTCHD1-AS PTENP1-AS PTGES2-AS1 PACERR PTOV1-AS1 PTOV1-AS2 PTPRD-AS1 PTPRD-AS2 PTPRG-AS1 PTPRJ-AS1 LOC101060632 LOC101926984 LOC151760 LOC100507556 PVRL3-AS1 PVT1 PXN-AS1 PYCARD-AS1 RAB11B-AS1 RAB30-AS1 RAB4B-EGLN2 RAB6C-AS1 RAD21-AS1 RAD51-AS1 RAD51L3-RFFL RAET1E-AS1 RAI1-AS1 RALY-AS1 RAMP2-AS1 RAP2C-AS1 RAPGEF4-AS1 RARA-AS1 RASA2-IT1 RASA3-IT1 RASAL2-AS1 RASGRF2-AS1 RASSF1-AS1 RASSF8-AS1 RBAKDN RBFADN RBM12B-AS1 RBM26-AS1 RBM5-AS1 RBMS3-AS1 RBMS3-AS2 RBMS3-AS3 RBPMS-AS1 RCAN3AS RDH10-AS1 RERG-AS1 RERG-IT1 RBSG2 RFPL1S RFPL3S RFX3-AS1 RGMB-AS1 RGPD4-AS1 RMST RHOXF1-AS1 RHPN1-AS1 RPPH1 RMDN2-AS1 RMRP RN7SL1 RN7SL2 RNY1

RNY3 RNY4 RNY5 RNASEH1-AS1 RNASEH2B-AS1 RNASEK-C17orf49 RNF139-AS1 RNF144A-AS1 RNF157-AS1 RNF185-AS1 RNF216-IT1 RNF217-AS1 RNF219-AS1 ROPN1L-AS1 ROR1-AS1 RORA-AS1 RORA-AS2 RORB-AS1 RPARP-AS1 RPL34-AS1 RPS6KA2-AS1 RPS6KA2-IT1 RRS1-AS1 RSF1-IT1 RSF1-IT2 RTCA-AS1 RTEL1-TNFRSF6B RUNDC3A-AS1 RUNX1-IT1 RUVBL1-AS1 SACS-AS1 SAMD12-AS1 SAMSN1-AS1 SAP30L-AS1 SAPCD1-AS1 SATB1-AS1 SATB2-AS1 SBF2-AS1 SCAANT1 SCAMP1-AS1 SCEL-AS1 SCOC-AS1 SDCBP2-AS1 SEC24B-AS1 SEMA3B-AS1 SEMA6A-AS1 SALRNA1 SALRNA2 SALRNA3 SENP3-EIF4A1 SEPSECS-AS1 SEPT4-AS1 SEPT5-GP1BB SEPT7-AS1 SERF2-C15ORF63 SERTAD4-AS1 SFTPD-AS1 SGMS1-AS1 SGOL1-AS1 SH3BP5-AS1 SH3PXD2A-AS1 SH3RF3-AS1 SHANK2-AS1 SHANK2-AS2 SHANK2-AS3 SIAH2-AS1 SIDT1-AS1 SIRPG-AS1 SIX3-AS1 LOC101928202 SLC14A2-AS1 SLC16A1-AS1 SLC16A12-AS1 SLC25A21-AS1 SLC25A25-AS1 SLC25A30-AS1 SLC25A5-AS1 SLC26A4-AS1 SLC2A1-AS1 SLC39A12-AS1 SLC6A1-AS1 SLC7A11-AS1 SLC8A1-AS1 SLC9A9-AS1 SLCO4A1-AS1 SLFNL1-AS1 SLIT1-AS1 SLIT2-IT1 SLMO2-ATP5E SLX1A-SULT1A3 SLX1B-SULT1A4 SMAD1-AS1 SMAD1-AS2 SMAD5-AS1 SMAD9-IT1 SCARNA1 SCARNA10 SCARNA11 SCARNA12 SCARNA13 SCARNA14 SCARNA15 SCARNA16 SCARNA17 SCARNA18 SCARNA2 SCARNA20 SCARNA21 SCARNA22 SCARNA23 SCARNA27 SCARNA3 SCARNA4 SCARNA5 SCARNA6 SCARNA7 SCARNA8 SCARNA9 SCARNA9L SMIM10L2A SMIM10L2B SNHG1 SNHG10 SNHG11 SNHG12 SNHG14 SNHG15 SNHG16 SNHG17 SNHG18 SNHG19 SNHG20 SNHG21 SNHG22 SNHG23 SNHG24 SNHG3 SNHG4 SNHG5 SNHG6 SNHG7 SNHG8 SNHG9 SMARCA5-AS1 SMC2-AS1 SMC5-AS1 SMG7-AS1 SMIM2-AS1 SMIM2-IT1 SMCR2 SMCR5 SMCR6 SENCR SNAI3-AS1 SNAP25-AS1 SNAP47-AS1 SNCA-AS1 SND1-IT1 SNRK-AS1 SOCS2-AS1 SORCS3-AS1 SOS1-IT1 SOX2-OT SOX21-AS1 SOX9-AS1 SP2-AS1 SPACA6P-AS SPAG5-AS1 SPANXA2-OT1 SPATA13-AS1 SPATA17-AS1 SPATA3-AS1 SPATA8-AS1 SPECC1L-ADORA2A SPACA6P SPATA41 SPATA42 SPG20-AS1 SPIN4-AS1 SFPQ SPRY4-IT1 SPTY2D1-AS1 SRD5A3-AS1 SRGAP2-AS1 SRGAP3-AS1 SRGAP3-AS2 SRGAP3-AS3 SRGAP3-AS4 SRP14-AS1 SRP54-AS1 SRRM2-AS1 SSBP3-AS1 SSSCA1-AS1 SSTR5-AS1 ST20-AS1 ST3GAL4-AS1 ST3GAL5-AS1 ST3GAL6-AS1 ST6GAL2-IT1 ST7-AS1 ST7-AS2 ST7-OT3 ST7-OT4 ST8SIA6-AS1 STAG3L5P-PVRIG2P-PILRB STAM-AS1 STARD13-AS STARD13-IT1 STARD4-AS1 STARD7-AS1 STAU2-AS1 STEAP2-AS1 STEAP3-AS1 STK24-AS1 STK4-AS1 STPG2-AS1 STX16-NPEPL1 STX17-AS1 STX18-AS1 STX18-IT1 STXBP5-AS1 SUCLA2-AS1 SUCLG2-AS1 SVIL-AS1 SCHLAP1 SYNE1-AS1 SYNJ2-IT1 SYNPR-AS1 SYP-AS1 SYS1-DBNDD2 SZT2-AS1 TRG-AS1 TAB3-AS2 TAF1A-AS1 TAPT1-AS1 TAT-AS1 TUG1 TBC1D22A-AS1 TBL1XR1-AS1 TBX2-AS1 TBX5-AS1 TCEAL3-AS1 TCEB3-AS1 TCL6 TCERG1L-AS1 TARID TCF7L1-IT1 TUNAR TERC TEN1-CDK3 TESC-AS1 TDRG1 TEX41 TSL TTTY1 TTTY10 TTTY11 TTTY12 TTTY13 TTTY13B TTTY14 TTTY15 TTTY16 TTTY17A TTTY17B TTTY17C TTTY18 TTTY19 TTTY1B TTTY2 TTTY20 TTTY21 TTTY21B TTTY22 TTTY23 TTTY23B TTTY2B TTTY3 TTTY3B TTTY4 TTTY4B TTTY4C TTTY5 TTTY6 TTTY6B TTTY7 TTTY7B TTTY8 TTTY8B TTTY9A TTTY9B TET2-AS1 TEX26-AS1 TEX36-AS1 TFAP2A-AS1 TGFA-IT1 TGFB2-AS1 TGFB2-OT1 THAP7-AS1 THAP9-AS1 THOC7-AS1 THRB-AS1 THRB-IT1 THSD4-AS1 THSD4-AS2 THUMPD3-AS1 THCAT126 TIPARP-AS1 TINCR TLR8-AS1 TLX1NB TM4SF1-AS1 TM4SF19-AS1 TM4SF19-TCTEX1D2 TMC3-AS1 TMCC1-AS1 TMEM108-AS1 TMEM147-AS1 TMEM161B-AS1 TMEM212-AS1 TMEM212-IT1 TMEM220-AS1 TMEM246-AS1 TMEM254-AS1 TMEM256-PLSCR3 TMEM26-AS1 TMEM44-AS1 TMEM5-AS1 TMEM51-AS1 TMEM72-AS1 TMEM92-AS1 TMEM9B-AS1 TMLHE-AS1 TMPO-AS1 TMPRSS4-AS1 TMX2-CTNND1 THRIL TNKS2-AS1 TNR-IT1 TNRC6C-AS1 TOB1-AS1 TOLLIP-AS1 TONSL-AS1 TOPORS-AS1 TP53TG1 TP73-AS1 TPRG1-AS1 TPRG1-AS2 TPT1-AS1 TRAF3IP2-AS1 TRAM2-AS1 TRERNA1 TMEM75 TMEM78 TRAPPC12-AS1 TRHDE-AS1 TRIM31-AS1 TRIM36-IT1 TRIM52-AS1 TRPC7-AS1 TRPM2-AS TSC22D1-AS1 TSIX TSNAX-DISC1 TSPAN9-IT1 TSPEAR-AS1 TSPEAR-AS2 TSSC1-IT1 TTC21B-AS1 TTC28-AS1 TTC3-AS1 TTC39A-AS1 TTC39C-AS1 TTLL7-IT1 TTN-AS1 TUB-AS1 TP53COR1 TUSC7 TUSC8 TSG1 TXNDC12-AS1 UBA6-AS1 UBAC2-AS1 UBE2E1-AS1 UBE2F-SCLY UBE2Q1-AS1 UBE2R2-AS1 UBL7-AS1 UBOX5-AS1 UBR5-AS1 UBXN10-AS1 UBXN7-AS1 UCHL1-AS1 UCKL1-AS1 UFL1-AS1 UGDH-AS1 UMODL1-AS1 UNC5B-AS1 RNR2 16S rRNA 16S rRNA RNA18S5 RNA28S5 RNA45S5 RNA5-8S5 RNA5S1 RNA5S10 RNA5S11 RNA5S12 RNA5S13 RNA5S14 RNA5S15 RNA5S16 RNA5S17 RNA5S2 RNA5S3 RNA5S4 RNA5S5 RNA5S6 RNA5S7 RNA5S8 RNA5S9 RNR1 12S rRNA 12S rRNA RNU105B RNU105C RNU86 SNORD10 SNORD100 SNORD101 SNORD102 SNORD103A SNORD103B SNORD104 SNORD105 SNORD105B SNORD107 SNORD108 SNORD109A SNORD109B SNORD11 SNORD110 SNORD111 SNORD111B SNORD112 SNORD113-1 SNORD113-2 SNORD113-3 SNORD113-4 SNORD113-5 SNORD113-6 SNORD113-7 SNORD113-8 SNORD113-9 SNORD114-1 SNORD114-10 SNORD114-11 SNORD114-12 SNORD114-13 SNORD114-14 SNORD114-15 SNORD114-16 SNORD114-17 SNORD114-18 SNORD114-19 SNORD114-2 SNORD114-20 SNORD114-21 SNORD114-22 SNORD114-23 SNORD114-24 SNORD114-25 SNORD114-26 SNORD114-27 SNORD114-28 SNORD114-29

SNORD114-3 SNORD114-30 SNORD114-31 SNORD114-4 SNORD114-5 SNORD114-6 SNORD114-7 SNORD114-8 SNORD114-9 SNORD115-1 SNORD115-10 SNORD115-11 SNORD115-12 SNORD115-13 SNORD115-14 SNORD115-15 SNORD115-16 SNORD115-17 SNORD115-18 SNORD115-19 SNORD115-2 SNORD115-20 SNORD115-21 SNORD115-22 SNORD115-23 SNORD115-24 SNORD115-25 SNORD115-26 SNORD115-27 SNORD115-28 SNORD115-29 SNORD115-3 SNORD115-30 SNORD115-31 SNORD115-32 SNORD115-33 SNORD115-34 SNORD115-35 SNORD115-36 SNORD115-37 SNORD115-38 SNORD115-39 SNORD115-4 SNORD115-40 SNORD115-41 SNORD115-42 SNORD115-43 SNORD115-44 SNORD115-45 SNORD115-46 SNORD115-47 SNORD115-48 SNORD115-5 SNORD115-6 SNORD115-7 SNORD115-8 SNORD115-9 SNORD116-1 SNORD116-10 SNORD116-11 SNORD116-12 SNORD116-13 SNORD116-14 SNORD116-15 SNORD116-16 SNORD116-17 SNORD116-18 SNORD116-19 SNORD116-2 SNORD116-20 SNORD116-21 SNORD116-22 SNORD116-23 SNORD116-24 SNORD116-25 SNORD116-26 SNORD116-27 SNORD116-28 SNORD116-29 SNORD116-3 SNORD116-30 SNORD116-4 SNORD116-5 SNORD116-6 SNORD116-7 SNORD116-8 SNORD116-9 SNORD117 SNORD118 SNORD119 SNORD11B SNORD12 SNORD121A SNORD121B SNORD123 SNORD124 SNORD125 SNORD126 SNORD127 SNORD12B SNORD12C SNORD13 SNORD14A SNORD14B SNORD14C SNORD14D SNORD14E SNORD15A SNORD15B SNORD16 SNORD17 SNORD18A SNORD18B SNORD18C SNORD19 SNORD19B SNORD1A SNORD1B SNORD1C SNORD2 SNORD20 SNORD21 SNORD22 SNORD23 SNORD24 SNORD25 SNORD26 SNORD27 SNORD28 SNORD29 SNORD30 SNORD31 SNORD32A SNORD32B SNORD33 SNORD34 SNORD35A SNORD35B SNORD36A SNORD36B SNORD36C SNORD37 SNORD38A SNORD38B SNORD3A SNORD3B-1 SNORD3B-2 SNORD3C SNORD3D SNORD41 SNORD42A SNORD42B SNORD43 SNORD44 SNORD45A SNORD45B SNORD45C SNORD46 SNORD47 SNORD48 SNORD49A SNORD49B SNORD4A SNORD4B SNORD5 SNORD50A SNORD50B SNORD51 SNORD52 SNORD53 SNORD54 SNORD55 SNORD56 SNORD56B SNORD57 SNORD58A SNORD58B SNORD58C SNORD59A SNORD59B SNORD6 SNORD60 SNORD61 SNORD62A SNORD62B SNORD63 SNORD64 SNORD65 SNORD66 SNORD67 SNORD68 SNORD69 SNORD7 SNORD70 SNORD71 SNORD72 SNORD73A SNORD74 SNORD75 SNORD76 SNORD77 SNORD78 SNORD79 SNORD8 SNORD80 SNORD81 SNORD82 SNORD83A SNORD83B SNORD84 SNORD85 SNORD86 SNORD87 SNORD88A SNORD88B SNORD88C SNORD89 SNORD9 SNORD90 SNORD91A SNORD91B SNORD92 SNORD93 SNORD94 SNORD95 SNORD96A SNORD96B SNORD97 SNORD98 SNORD99 SNORA1 SNORA10 SNORA11 SNORA11B SNORA11C SNORA11D SNORA11E SNORA12 SNORA13 SNORA14A SNORA14B SNORA15 SNORA16A SNORA16B SNORA17 SNORA18 SNORA19 SNORA20 SNORA21 SNORA22 SNORA23 SNORA24 SNORA25 SNORA26 SNORA27 SNORA28 SNORA29 SNORA2A SNORA2B SNORA30 SNORA31 SNORA32 SNORA33 SNORA34 SNORA35 SNORA36A SNORA36B SNORA36C SNORA37 SNORA38 SNORA38B SNORA4 SNORA40 SNORA41 SNORA43 SNORA44 SNORA45A SNORA45B SNORA46 SNORA47 SNORA48 SNORA49 SNORA51 SNORA52 SNORA53 SNORA54 SNORA55 SNORA56 SNORA57 SNORA58 SNORA59A SNORA59B SNORA5A SNORA5B SNORA5C SNORA6 SNORA60 SNORA61 SNORA62 SNORA63 SNORA64 SNORA65 SNORA66 SNORA67 SNORA68 SNORA69 SNORA70 SNORA70B SNORA70C SNORA70D SNORA70E SNORA70F SNORA70G SNORA71A SNORA71B SNORA71C SNORA71D SNORA71E SNORA72 SNORA73A SNORA73B SNORA74A SNORA74B SNORA75 SNORA76A SNORA76C SNORA77 SNORA78 SNORA79 SNORA7A SNORA7B SNORA8 SNORA80A SNORA80B SNORA80E SNORA81 SNORA84 SNORA9 RN7SK RNU1-1 RNU1-13P RNU1-2 RNU1-27P RNU1-28P RNU1-3 RNU1-4 RNU11 RNU12 RNU2-1 RNU2-2P RNU4-1 RNU4-2 RNU4ATAC RNU5A-1 RNU5B-1 RNU5D-1 RNU5E-1 RNU5F-1 RNU6-1 RNU6-10P RNU6-14P RNU6-15P RNU6-16P RNU6-19P RNU6-2 RNU6-21P RNU6-23P RNU6-26P RNU6-28P RNU6-30P RNU6-31P RNU6-33P RNU6-34P RNU6-35P RNU6-36P RNU6-39P RNU6-42P RNU6-45P RNU6-46P RNU6-48P RNU6-52P RNU6-53P RNU6-55P RNU6-56P RNU6-57P RNU6-58P RNU6-59P RNU6-63P RNU6-64P RNU6-66P RNU6-67P RNU6-68P RNU6-69P RNU6-7 RNU6-71P RNU6-72P RNU6-75P RNU6-76P RNU6-78P RNU6-79P RNU6-8 RNU6-81P RNU6-82P RNU6-83P RNU6-9 RNU6ATAC RNU7-1 RNVU1-1 RNVU1-14 RNVU1-15 RNVU1-17 RNVU1-18 RNVU1-19 RNVU1-20 RNVU1-3 RNVU1-4 RNVU1-6 RNVU1-7 RNVU1-8 SNAR-A1 SNAR-A10 SNAR-A11 SNAR-A12 SNAR-A13 SNAR-A14 SNAR-A2 SNAR-A3 SNAR-A4 SNAR-A5 SNAR-A6 SNAR-A7 SNAR-A8 SNAR-A9 SNAR-B1 SNAR-B2 SNAR-C1 SNAR-C2 SNAR-C3 SNAR-C4 SNAR-C5 SNAR-D SNAR-E SNAR-F SNAR-G1 SNAR-G2 SNAR-H SNAR-I NMTRQ-TTG14-1 NMTRQ-TTG3-1 NMTRQ-TTG5-1 NMTRL-TAA1-1 NMTRL-TAA4-1 NMTRS-TGA1-1 TRNAA-AGC TRNAA-CGC TRNAA-UGC TRR TRNAR-ACG TRNAR-CCG TRNAR-CCU TRNAR-UCG TRNAR-UCU TRNAN-GUU TRNAD-GUC TRNAC-GCA TRNAE-CUC TRNAE-UUC TRNAQ-CUG TRNAQ-UUG TRNAG-CCC TRNAG-GCC TRNAG-UCC TRNAH-GUG TRNAI-AAU TRNAI-GAU TRNAI-UAU TRNAL-AAG TRNAL-CAA TRNAL-CAG TRNAL-UAA TRNAL-UAG TRNAK-CUU TRNAK-UUU TRNAM-CAU TRNASTOP-UUA

TRNASTOP-UCA TRNAF-GAA TRNAP-AGG TRNAP-CGG TRNAP-UGG TRNAS-AGA TRNAS-CGA TRNAS-GCU TRNAS-UGA TRSUP-CTA1-1 TRSUP-TTA1-1 TRSUP-TTA2-1 TRNAT-AGU TRNAT-CGU TRNAT-UGU TRNAW-CCA TRNAY-AUA TRNAY-GUA TRNAV-AAC TRNAV-CAC TRNAV-UAC TRA-AGC10-1 TRA-AGC1-1 TRA-AGC11-1 TRA-AGC12-1 TRA-AGC12-2 TRA-AGC12-3 TRA-AGC13-1 TRA-AGC13-2 TRA-AGC14-1 TRA-AGC15-1 TRA-AGC16-1 TRA-AGC17-1 TRA-AGC18-1 TRA-AGC18-2 TRA-AGC19-1 TRA-AGC20-1 TRA-AGC2-1 TRA-AGC21-1 TRA-AGC2-2 TRA-AGC22-1 TRA-AGC3-1 TRA-AGC4-1 TRA-AGC5-1 TRA-AGC6-1 TRA-AGC7-1 TRA-AGC8-1 TRA-AGC8-2 TRA-AGC9-1 TRA-AGC9-2 TRA-CGC1-1 TRA-CGC2-1 TRA-CGC3-1 TRA-CGC4-1 TRA-CGC5-1 TRA-TGC1-1 TRA-TGC2-1 TRA-TGC3-1 TRA-TGC3-2 TRA-TGC4-1 TRA-TGC5-1 TRA-TGC6-1 TRA-TGC7-1 TRA-TGC8-1 TRR-ACG1-1 TRR-ACG1-2 TRR-ACG1-3 TRR-ACG2-1 TRR-ACG2-2 TRR-ACG2-3 TRR-ACG2-4 TRR-CCG1-1 TRR-CCG1-2 TRR-CCG1-3 TRR-CCG2-1 TRR-CCT1-1 TRR-CCT2-1 TRR-CCT3-1 TRR-CCT4-1 TRR-CCT5-1 TRR-TCG1-1 TRR-TCG2-1 TRR-TCG3-1 TRR-TCG4-1 TRR-TCG5-1 TRR-TCG6-1 TRR-TCT1-1 TRR-TCT2-1 TRR-TCT3-1 TRR-TCT3-2 TRR-TCT4-1 TRR-TCT5-1 TRN-ATT1-1 TRN-ATT1-2 TRN-GTT10-1 TRN-GTT1-1 TRN-GTT11-1 TRN-GTT11-2 TRN-GTT12-1 TRN-GTT13-1 TRN-GTT14-1 TRN-GTT15-1 TRN-GTT16-1 TRN-GTT16-2 TRN-GTT16-3 TRN-GTT16-4 TRN-GTT16-5 TRN-GTT17-1 TRN-GTT18-1 TRN-GTT19-1 TRN-GTT20-1 TRN-GTT2-1 TRN-GTT21-1 TRN-GTT2-2 TRN-GTT2-3 TRN-GTT2-4 TRN-GTT2-5 TRN-GTT2-6 TRN-GTT3-1 TRN-GTT3-2 TRN-GTT4-1 TRN-GTT5-1 TRN-GTT6-1 TRN-GTT7-1 TRN-GTT8-1 TRN-GTT9-1 TRN-GTT9-2 TRD-GTC1-1 TRD-GTC2-1 TRD-GTC2-10 TRD-GTC2-11 TRD-GTC2-2 TRD-GTC2-3 TRD-GTC2-4 TRD-GTC2-5 TRD-GTC2-6 TRD-GTC2-7 TRD-GTC2-8 TRD-GTC2-9 TRD-GTC3-1 TRD-GTC4-1 TRD-GTC5-1 TRD-GTC6-1 TRD-GTC7-1 TRD-GTC8-1 TRD-GTC9-1 TRC-GCA10-1 TRC-GCA1-1 TRC-GCA11-1 TRC-GCA12-1 TRC-GCA13-1 TRC-GCA14-1 TRC-GCA15-1 TRC-GCA16-1 TRC-GCA17-1 TRC-GCA18-1 TRC-GCA19-1 TRC-GCA20-1 TRC-GCA2-1 TRC-GCA21-1 TRC-GCA2-2 TRC-GCA22-1 TRC-GCA2-3 TRC-GCA23-1 TRC-GCA2-4 TRC-GCA24-1 TRC-GCA3-1 TRC-GCA4-1 TRC-GCA5-1 TRC-GCA6-1 TRC-GCA7-1 TRC-GCA8-1 TRC-GCA9-1 TRC-GCA9-2 TRC-GCA9-3 TRC-GCA9-4 TRQ-CTG10-1 TRQ-CTG1-1 TRQ-CTG1-2 TRQ-CTG12-1 TRQ-CTG1-3 TRQ-CTG1-4 TRQ-CTG14-1 TRQ-CTG1-5 TRQ-CTG15-1 TRQ-CTG17-1 TRQ-CTG18-1 TRQ-CTG2-1 TRQ-CTG3-1 TRQ-CTG3-2 TRQ-CTG4-1 TRQ-CTG4-2 TRQ-CTG5-1 TRQ-CTG6-1 TRQ-CTG7-1 TRQ-CTG8-1 TRQ-CTG8-2 TRQ-TTG10-1 TRQ-TTG1-1 TRQ-TTG2-1 TRQ-TTG3-1 TRQ-TTG3-2 TRQ-TTG3-3 TRQ-TTG4-1 TRQ-TTG6-1 TRE-CTC1-1 TRE-CTC1-2 TRE-CTC1-3 TRE-CTC1-4 TRE-CTC1-5 TRE-CTC1-6 TRE-CTC1-7 TRE-CTC17-1 TRE-CTC2-1 TRE-CTC3-1 TRE-CTC5-1 TRE-CTC6-1 TRE-CTC8-1 TRE-TTC1-1 TRE-TTC11-1 TRE-TTC1-2 TRE-TTC12-1 TRE-TTC13-1 TRE-TTC16-1 TRE-TTC2-1 TRE-TTC2-2 TRE-TTC3-1 TRE-TTC4-1 TRE-TTC4-2 TRE-TTC5-1 TRE-TTC8-1 TRG-CCC1-1 TRG-CCC1-2 TRG-CCC2-1 TRG-CCC2-2 TRG-CCC3-1 TRG-CCC5-1 TRG-CCC8-1 TRG-GCC1-1 TRG-GCC1-2 TRG-GCC1-3 TRG-GCC1-4 TRG-GCC1-5 TRG-GCC2-1 TRG-GCC2-2 TRG-GCC2-3 TRG-GCC2-4 TRG-GCC2-5 TRG-GCC2-6 TRG-GCC3-1 TRG-GCC4-1 TRG-GCC5-1 TRG-GCC6-1 TRG-TCC1-1 TRG-TCC2-1 TRG-TCC2-2 TRG-TCC2-3 TRG-TCC2-4 TRG-TCC2-5 TRG-TCC2-6 TRG-TCC3-1 TRG-TCC4-1 TRH-GTG1-1 TRH-GTG1-2 TRH-GTG1-3 TRH-GTG1-4 TRH-GTG1-5 TRH-GTG1-6 TRH-GTG1-7 TRH-GTG1-8 TRH-GTG1-9 TRH-GTG2-1 TRH-GTG3-1 TRI-AAT1-1 TRI-AAT2-1 TRI-AAT3-1 TRI-AAT4-1 TRI-AAT5-1 TRI-AAT5-2 TRI-AAT5-3 TRI-AAT5-4 TRI-AAT5-5 TRI-AAT6-1 TRI-AAT7-1 TRI-AAT7-2 TRI-AAT8-1 TRI-AAT9-1 TRI-GAT1-1 TRI-GAT1-2 TRI-GAT1-3 TRI-TAT1-1 TRI-TAT2-1 TRI-TAT2-2 TRI-TAT2-3 TRI-TAT3-1 TRX-CAT1-1 TRX-CAT1-2 TRX-CAT1-3 TRX-CAT1-4 TRX-CAT1-5 TRX-CAT1-6 TRX-CAT1-7 TRX-CAT1-8 TRX-CAT2-1 TRX-CAT3-1 TRL-AAG1-1 TRL-AAG1-2 TRL-AAG1-3 TRL-AAG2-1 TRL-AAG2-2 TRL-AAG2-3 TRL-AAG2-4 TRL-AAG3-1 TRL-AAG4-1 TRL-AAG5-1 TRL-AAG6-1 TRL-AAG8-1 TRL-CAA1-1 TRL-CAA1-2 TRL-CAA2-1 TRL-CAA3-1 TRL-CAA4-1 TRL-CAA5-1 TRL-CAA6-1 TRL-CAG1-1 TRL-CAG1-2 TRL-CAG1-3 TRL-CAG1-4 TRL-CAG1-5 TRL-CAG1-6 TRL-CAG1-7 TRL-CAG2-1 TRL-CAG2-2 TRL-CAG3-1 TRL-TAA1-1 TRL-TAA2-1 TRL-TAA3-1 TRL-TAA4-1 TRL-TAA5-1 TRL-TAG1-1 TRL-TAG2-1 TRL-TAG3-1 TRK-CTT10-1 TRK-CTT1-1 TRK-CTT11-1 TRK-CTT1-2 TRK-CTT15-1 TRK-CTT2-1 TRK-CTT2-2 TRK-CTT2-3 TRK-CTT2-4 TRK-CTT2-5 TRK-CTT3-1 TRK-CTT4-1 TRK-CTT5-1 TRK-CTT6-1 TRK-CTT8-1 TRK-CTT9-1 TRK-TTT1-1 TRK-TTT11-1 TRK-TTT12-1 TRK-TTT16-1 TRK-TTT2-1 TRK-TTT3-1 TRK-TTT3-2 TRK-TTT3-3 TRK-TTT3-4 TRK-TTT3-5 TRK-TTT4-1 TRK-TTT5-1 TRK-TTT6-1 TRK-TTT7-1 TRK-TTT8-1 TRK-TTT9-1 TRM-CAT1-1 TRM-CAT2-1 TRM-CAT3-1 TRM-CAT3-2 TRM-CAT4-1 TRM-CAT4-2 TRM-CAT4-3 TRM-CAT5-1 TRM-CAT6-1 TRM-CAT7-1 TRF-GAA1-1 TRF-GAA1-2 TRF-GAA1-3 TRF-GAA1-4 TRF-GAA1-5 TRF-GAA1-6 TRF-GAA2-1 TRF-GAA3-1 TRF-GAA4-1 TRF-GAA5-1 TRF-GAA6-1 TRF-GAA7-1 TRP-AGG1-1 TRP-AGG2-1 TRP-AGG2-2 TRP-AGG2-3 TRP-AGG2-4 TRP-AGG2-5 TRP-AGG2-6 TRP-AGG2-7 TRP-AGG2-8 TRP-AGG3-1 TRP-CGG1-1 TRP-CGG1-2 TRP-CGG1-3 TRP-CGG2-1 TRP-TGG1-1 TRP-TGG2-1 TRP-TGG3-1 TRP-TGG3-2 TRP-TGG3-3 TRP-TGG3-4 TRP-TGG3-5 TRU-TCA1-1 TRU-TCA2-1 TRU-TCA3-1 TRS-AGA1-1 TRS-AGA2-1 TRS-AGA2-2 TRS-AGA2-3

TRS-AGA2-4 TRS-AGA2-5 TRS-AGA2-6 TRS-AGA3-1 TRS-AGA4-1 TRS-AGA5-1 TRS-AGA6-1 TRS-CGA1-1 TRS-CGA2-1 TRS-CGA3-1 TRS-CGA4-1 TRS-GCT1-1 TRS-GCT2-1 TRS-GCT3-1 TRS-GCT4-1 TRS-GCT4-2 TRS-GCT4-3 TRS-GCT5-1 TRS-GCT6-1 TRS-TGA1-1 TRS-TGA2-1 TRS-TGA3-1 TRS-TGA4-1 TRT-AGT1-1 TRT-AGT1-2 TRT-AGT1-3 TRT-AGT2-1 TRT-AGT2-2 TRT-AGT3-1 TRT-AGT4-1 TRT-AGT5-1 TRT-AGT6-1 TRT-AGT7-1 TRT-CGT1-1 TRT-CGT2-1 TRT-CGT3-1 TRT-CGT4-1 TRT-CGT5-1 TRT-CGT6-1 TRT-TGT1-1 TRT-TGT2-1 TRT-TGT3-1 TRT-TGT4-1 TRT-TGT5-1 TRT-TGT6-1 TRW-CCA1-1 TRW-CCA2-1 TRW-CCA3-1 TRW-CCA3-2 TRW-CCA3-3 TRW-CCA4-1 TRW-CCA5-1 TRW-CCA6-1 TRW-CCA7-1 TRY-ATA1-1 TRY-GTA10-1 TRY-GTA1-1 TRY-GTA2-1 TRY-GTA3-1 TRY-GTA4-1 TRY-GTA5-1 TRY-GTA5-2 TRY-GTA5-3 TRY-GTA5-4 TRY-GTA5-5 TRY-GTA6-1 TRY-GTA7-1 TRY-GTA8-1 TRY-GTA9-1 TRV-AAC1-1 TRV-AAC1-2 TRV-AAC1-3 TRV-AAC1-4 TRV-AAC1-5 TRV-AAC2-1 TRV-AAC3-1 TRV-AAC4-1 TRV-AAC5-1 TRV-AAC6-1 TRV-AAC7-1 TRV-CAC10-1 TRV-CAC1-1 TRV-CAC11-1 TRV-CAC1-2 TRV-CAC12-1 TRV-CAC1-3 TRV-CAC1-4 TRV-CAC1-5 TRV-CAC1-6 TRV-CAC2-1 TRV-CAC3-1 TRV-CAC4-1 TRV-CAC5-1 TRV-CAC6-1 TRV-CAC7-1 TRV-CAC8-1 TRV-CAC9-1 TRV-TAC1-1 TRV-TAC1-2 TRV-TAC2-1 TRV-TAC3-1 TRV-TAC4-1 TRNC TRNA TRND TRNE TRNF TRNG TRNH TRNI TRNK TRNL1 TRNL2 TRNM TRNN TRNP TRNQ TRNR TRNS1 TRNS2 TRNT TRNV TRNW TRNY trnT trnE trnL trnS trnH trnR trnG trnK trnS trnD trnY trnC trnL trnF trnP trnV trnN trnW trnA trnQ trnM trnI trnF trnV trnL trnS trnK trnG trnT trnI trnW trnR trnH trnE trnC trnY trnM trnS trnQ trnL trnD trnP trnA TRNAG1

Table 13. Cell surface markers in maturing erythroid cells

Stage	Markers			
	GPA-positive	Alpha4 integrin-positive	Band3-positive	Alpha4 integrin-positive and Band3-positive
M0	83.9%	98.0%	54.6%	52.9%
M3	99.0%	91.4%	97.8%	89.6%
M5	99.5%	84.2%	100%	84.2%

Table 14. Co-expression of EGFP and mCherry

Stage	Percent cells positive for:		
	EGFP only	mCherry only	EGFP and mCherry
M6	90.4%	89.05%	86.55%
M11	77.75%	75.90%	66.05%
M13	86.00%	80.30%	75.40%
M18	94.15%	90.80%	86.40%

Table 15: Co-expression of 4-1BBL and Avelumab

Sample	Percent cells positive for:		
	4-1BBL	Avelumab	4-1BBL and Avelumab
Negative control	0.99%	0.24%	0.035%
Erythroid cells + m4-1BBL only	92.5%	0.58%	0.39%
Erythroid cells + Avelumab only	1.4%	75.0%	0.59%
Erythroid cells + m4-	61.4%	70.9%	58.5%

1BBL and avelumab			

Table 16: Dose expression results

Amount mRNA added (mg)	Percent of cells expressing 4-1BBL	Number of copies of 4-1BBL per cell
0.6	87.5%	1,015,250
0.4	90.6%	874,017
0.2	92.0%	609,145
0.1	91.75%	274,766
0.05	87.7%	100,500
0.025	74.0%	42,902
0	1.25%	NA

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

What is claimed is:

1. A method of making an erythroid cell comprising an mRNA encoding an exogenous protein, comprising:

a) providing an erythroid cell in maturation phase, and

b) contacting the erythroid cell with an mRNA encoding the exogenous protein, under conditions that allow uptake of the mRNA by the erythroid cell,

thereby making an erythroid cell comprising an mRNA encoding an exogenous protein.

2. The method of claim 1, wherein the erythroid cell takes up the mRNA encoding the exogenous protein.

3. The method of claim 1, comprising providing a population of erythroid cells in maturation phase and contacting a plurality of cells of the population of erythroid cells with the mRNA encoding the exogenous protein.

4. The method of claim 3, wherein the plurality of cells of the population of erythroid cells each takes up the mRNA encoding the exogenous protein.

5. The method of any of claims 1-4, wherein after uptake of the mRNA encoding the exogenous protein, the cell or the plurality of cells express the exogenous protein.

6. The method of claim 5, wherein the cell or the plurality of cells comprise the exogenous protein.

7. The method of any of claims 3-6, wherein the population of erythroid cells in maturation phase is a population of cells expanded in a maturation medium for 3-7 days, e.g., 4-5 or 4-6 days.

8. A method of manufacturing a population of reticulocytes that express an exogenous protein, the method comprising

(e) providing a population of erythroid precursor cells (e.g., CD34+ cells);

(f) culturing the population of erythroid precursor cells under differentiating conditions to provide a population of differentiating erythroid cells;

(g) contacting a plurality of cells of the population of differentiating erythroid cells with an mRNA encoding the exogenous protein, under conditions that allow uptake of the mRNA by the plurality of cells of the population of differentiating erythroid cells; and

(h) further culturing the plurality of cells of the population of differentiating erythroid cells to provide a population of reticulocytes,

thereby manufacturing a population of reticulocytes that express the exogenous protein.

9. The method of claim 8, wherein the further culturing comprises fewer than 3, 2, or 1 population doubling.

10. The method of any of claims 3-9, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or more) of the following properties:

i.a) 2-40%, 3-33%, 5-30%, 10-25%, or 15-20% of the cells in the population are enucleated;

i.b) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 2%, 3%, 4%, or 5% of the cells in the population are enucleated;

i.c) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 6%, 10%, 15%, 20%, or 25% of the cells in the population are enucleated;

i.d) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 30%, 35%, 40%, 45%, or 50% of the cells in the population are enucleated;

i.e) no more than 1%, 2%, 3%, 5%, 10%, 15%, or 20% of the cells in the population are enucleated;

i.f) no more than 25%, 30%, 35%, 40%, 45%, or 50% of the cells in the population are enucleated;

i.g) the population of cells has reached 6-70%, 10-60%, 20-50%, or 30-40% of maximal enucleation;

i.h) the population of cells has reached no more than 1%, 2%, 3%, 5%, 10%, 15%, or 20% of maximal enucleation;

i.i) the population of cells has reached no more than 25%, 30%, 35%, 40%, 45%, 50%, or 60% of maximal enucleation;

ii.a) the population of cells is fewer than 3, 2, or 1 population doubling from a plateau in cell division;

ii.b) the population of cells is capable of fewer than 3, 2, or 1 population doubling;

ii.c) the population will increase by no more than 1.5, 2, or 3 fold before the population reaches an enucleation level of at least 70% of cells in the population;

iii.a) at least 80%, 85%, 90%, 95%, or 99% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.b) at least 50%, 60%, 70%, 75%, or 79% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.c) 30-90%, 40-90%, 50-90%, 60-90%, or 70-90% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.d) at least 80%, 85%, 90%, 95%, or 99% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast);

iii.e) at least 50%, 60%, 70%, 75%, or 79% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast); or

iii.f) 30-90%, 40-90%, 50-90%, 60-90%, or 70-90% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast).

11. The method of claim 10, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i and a property from ii.

12. The method of claim 10, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i and a property from iii.

13. The method of claim 10, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from ii and a property from iii.

14. The method of claim 10, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i, a property from ii, and a property from iii.

15. The method of any of claims 3-14, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following property: 84-99%, 85-95%, or about 90% of the cells in the population are GPA-positive, e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10.

16. The method of any of claims 3-14, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following property: at least 84%, 85%, 90%, 95%, or 99% of the cells in the population are GPA-positive, e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10.

17. The method of any of claims 3-14, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following property: 54-99%, 55-98%, 60-95%, 65-90%, 70-85%, or 75-80% of the cells in the population are band3-positive, e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10.

18. The method of any of claims 3-14, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following property: at least 54%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% of the cells in the population are band3-positive, e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10.

19. The method of any of claims 3-14, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following property: 96-100%, 97-99%, or about 98% of the cells in the population are alpha4 integrin-positive, e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10.

20. The method of any of claims 3-14, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising the following property: at least 95%, 96%, 97%, 98%, or 99% of the cells in the population are alpha4 integrin-positive, e.g., as measured by a flow cytometry assay, e.g., a flow cytometry assay of Example 10.

21. The method of any of claims 3-20, wherein prior to or after contacting the plurality of cells with the mRNA encoding the exogenous protein, the plurality of cells are separated from the population of erythroid cells or the population of differentiating erythroid cells, e.g., the plurality of cells are separated from the population based on enucleation status (e.g., the plurality of cells are nucleated cells and the rest of the population are enucleated cells).

22. The method of any of claims 3-20, comprising prior to or after contacting the plurality of cells with the mRNA encoding the exogenous protein, synchronizing the population of erythroid cells or the population of differentiating erythroid cells, e.g., by arresting the growth, development, hemoglobin synthesis, or the process of enucleation of the population, e.g., by incubating the population with an inhibitor of enucleation (e.g., an inhibitor of histone deacetylase (HDAC), an inhibitor of mitogen-activated protein kinase (MAPK), an inhibitor of cyclin-dependent kinase (CDK), or a proteasome inhibitor).

23. The method of claim 22, wherein arresting occurs prior to enucleation of more than 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10% of the cells in the population.

24. A method of manufacturing a population of reticulocytes that express an exogenous protein, the method comprising:

(i) providing a population of erythroid precursor cells (e.g., CD34+ cells);

(j) culturing the population of erythroid precursor cells under differentiating conditions to provide a population of differentiating erythroid cells;

(k) contacting the differentiating erythroid cells with an mRNA encoding the exogenous protein, under conditions that allow uptake of the mRNA by the differentiating erythroid cells, wherein the contacting is performed when the population of differentiating erythroid cells is between 0.1 and 25% enucleated (e.g., between 0.1 and 20% enucleated, between 0.1 and 15% enucleated, between 0.1 and 12% enucleated, or between 0.1 and 10% enucleated); and

(l) further culturing the differentiating erythroid cells to provide a population of reticulocytes,

thereby manufacturing a population of reticulocytes that express the exogenous protein.

25. The method of claim 24, wherein the further culturing comprises fewer than 3, 2, or 1 population doubling.

26. The method of claim 24 or 25, wherein the contacting is performed when at least 50% (at least 60%, 70%, 75%, 80%, 90%, or 95%) of the differentiating erythroid cells exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast).

27. A method of manufacturing a population of reticulocytes that express an exogenous protein, comprising (a) providing a population of erythroid precursor cells, (b) culturing the population of erythroid precursor cells under differentiating conditions to provide a population of differentiating erythroid cells, (c) contacting the differentiating erythroid cells with an mRNA encoding the exogenous protein, wherein the improvement comprises: the contacting is performed when the population of differentiating erythroid cells is between 0.1 and 25% enucleated (e.g., between 0.1 and 20% enucleated, between 0.1 and 15% enucleated, between 0.1 and 12% enucleated, or between 0.1 and 10% enucleated).

28. The method of claim 27, wherein the contacting is performed when the population of differentiating erythroid cells has fewer than 3, 2, or 1 population doubling before a plateau in cell division.

29. The method of claim 27 or 28, wherein the contacting is performed when at least 50% (at least 60%, 70%, 75%, 80%, 90%, or 95%) of the differentiating erythroid cells exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast).

30. An erythroid cell, e.g., an enucleated erythroid cell, comprising:

an exogenous mRNA comprising a coding region operatively linked to a heterologous untranslated region (UTR), wherein the heterologous UTR comprises a regulatory element.

31. An erythroid cell, e.g., an enucleated erythroid cell, comprising an exogenous mRNA that comprises one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof.

32. A method of producing an erythroid cell, e.g., enucleated erythroid cell, comprising:

a) contacting an erythroid cell, e.g., a nucleated erythroid cell, with an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element, (e.g., isolated RNA or in vitro transcribed RNA), and

b) maintaining the contacted erythroid cell under conditions suitable for uptake of the exogenous mRNA,

thereby producing the erythroid cell, e.g., an enucleated erythroid cell.

33. A method of producing an erythroid cell, e.g., enucleated erythroid cell, comprising:

a) contacting an erythroid cell, e.g., a nucleated erythroid cell, with an exogenous mRNA comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof; and

b) maintaining the contacted erythroid cell under conditions suitable for uptake of the exogenous mRNA,

thereby producing the erythroid cell, e.g., an enucleated erythroid cell.

34. A method of producing an exogenous protein in an enucleated erythroid cell:

a) providing an erythroid cell, e.g., a nucleated erythroid cell, comprising an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element, (e.g., isolated RNA or in vitro transcribed RNA), and

b) culturing the erythroid cell under conditions suitable for production of the exogenous protein,

thereby producing the exogenous protein.

35. A method of producing an exogenous protein in an enucleated erythroid cell:

a) providing an erythroid cell, e.g., a nucleated erythroid cell, comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof, and

b) culturing the erythroid cell under conditions suitable for production of the exogenous protein,

thereby producing the exogenous protein.

36. A method of providing a subject with an exogenous protein, providing a subject with an enucleated erythroid cell which can produce an exogenous protein, or treating a subject, comprising administering to the subject:

an erythroid cell, e.g., a nucleated erythroid cell, comprising an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element, (e.g., isolated RNA or in vitro transcribed RNA),

thereby providing a subject with an exogenous protein, providing a subject with an enucleated erythroid cell which can produce an exogenous protein, or treating a subject.

37. A method of providing a subject with an exogenous protein, providing a subject with an enucleated erythroid cell which can produce an exogenous protein, or treating a subject, comprising administering to the subject:

an erythroid cell, e.g., a nucleated erythroid cell, comprising an exogenous mRNA comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof,

thereby providing a subject with an exogenous protein, providing a subject with an enucleated erythroid cell which can produce an exogenous protein, or treating a subject.

38. A method of evaluating an erythroid cell, e.g., enucleated erythroid cell (or a batch of such cells) comprising:

a) providing an erythroid cell, e.g., a nucleated erythroid cell, comprising an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element (or a batch of such cells), and

b) evaluating the erythroid cell, e.g., the nucleated erythroid cell (or batch of such cells) for a preselected parameter,

thereby evaluating the erythroid cell, e.g., enucleated erythroid cell (or a batch of such cells).

39. A method of evaluating an erythroid cell, e.g., enucleated erythroid cell (or a batch of such cells) comprising:

a) providing an erythroid cell, e.g., a nucleated erythroid cell, comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof, and

b) evaluating the erythroid cell, e.g., the nucleated erythroid cell (or batch of such cells) for a preselected parameter,

thereby evaluating the erythroid cell, e.g., enucleated erythroid cell (or a batch of such cells).

40. The method of claim 30, wherein at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells in the population comprise the exogenous protein, e.g., 5 days after contacting with the mRNA.

41. The method of claim 30, wherein the cells in the population comprise at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the exogenous protein, e.g., 5 days after the contacting with the mRNA.

42. The method of claim 30, wherein the cells comprise at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the exogenous protein for at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days after contacting with the mRNA.

43. A method of making an erythroid cell comprising an mRNA that encodes an exogenous protein, comprising:

providing a reaction mixture comprising an erythroid cell and an mRNA encoding the exogenous protein, under conditions which inhibit degradation of mRNA, e.g., by inclusion in the reaction mixture a ribonuclease inhibitor, and

maintaining the reaction mixture under conditions that allow uptake of the mRNA by the erythroid cell,

thereby making an erythroid cell comprising an mRNA encoding an exogenous protein.

44. The method of claim 43, comprising providing a population of erythroid cells and contacting the population with the mRNA encoding the exogenous protein.

45. The method of claim 43 or 44, wherein a plurality of erythroid cells of the population each takes up an mRNA encoding the exogenous protein.

46. The method of any of claims 43-45, wherein the cell or plurality of cells express the exogenous protein.

47. The method of any of claims 43-46, wherein the cell or plurality of cells comprise the exogenous protein.

48. The method of any of claims 43-47, which further comprises electroporating the cell or population of cells.

49. The method of any of claims 43-48, which further comprises contacting a population of erythroid cells with a ribonuclease inhibitor.

50. The method of any of claims 43-49, which comprises contacting the population of cells with the ribonuclease inhibitor before, during, or after contacting the cells with the mRNA.

51. The method of any of claims 43-50, which comprises contacting the cells with the ribonuclease inhibitor at day 4, 5, or 6 of maturation phase.

52. The method of any of claims 43-51, wherein the cell is in maturation phase.

53. The method of any of claims 43-52, which comprises contacting the cells with the ribonuclease inhibitor at a time when the cells comprise one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or more) of the following properties:

i.a) 2-40%, 3-33%, 5-30%, 10-25%, or 15-20% of the cells in the population are enucleated;

i.b) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 2%, 3%, 4%, or 5% of the cells in the population are enucleated;

i.c) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 6%, 10%, 15%, 20%, or 25% of the cells in the population are enucleated;

i.d) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 30%, 35%, 40%, 45%, or 50% of the cells in the population are enucleated;

i.e) no more than 1%, 2%, 3%, 5%, 10%, 15%, or 20% of the cells in the population are enucleated;

i.f) no more than 25%, 30%, 35%, 40%, 45%, or 50% of the cells in the population are enucleated;

i.g) the population of cells has reached 6-70%, 10-60%, 20-50%, or 30-40% of maximal enucleation;

i.h) the population of cells has reached no more than 1%, 2%, 3%, 5%, 10%, 15%, or 20% of maximal enucleation;

i.i) the population of cells has reached no more than 25%, 30%, 35%, 40%, 45%, 50%, or 60% of maximal enucleation;

ii.a) the population of cells is fewer than 3, 2, or 1 population doubling from a plateau in cell division;

ii.b) the population of cells is capable of fewer than 3, 2, or 1 population doubling;

ii.c) the population will increase by no more than 1.5, 2, or 3 fold before the population reaches an enucleation level of at least 70% of cells in the population;

iii.a) at least 80%, 85%, 90%, 95%, or 99% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.b) at least 50%, 60%, 70%, 75%, or 79% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.c) 30-90%, 40-90%, 50-90%, 60-90%, or 70-90% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.d) at least 80%, 85%, 90%, 95%, or 99% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast);

iii.e) at least 50%, 60%, 70%, 75%, or 79% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast); or

iii.f) 30-90%, 40-90%, 50-90%, 60-90%, or 70-90% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast).

54. The method of claim 53, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i and a property from ii.

55. The method of claim 53, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i and a property from iii.

56. The method of claim 53, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from ii and a property from iii.

57. The method of claim 53, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i, a property from ii, and a property from iii.

58. The method of any of claims 43-57, which comprises contacting the cells with the ribonuclease inhibitor at a time when (e.g., by a flow cytometry assay, e.g., a flow cytometry assay of Example 10) the cells comprise one or more (e.g., 2, 3, 4, 5, or more) of the following properties:

84-99%, 85-95%, or about 90% of the cells in the population are GPA-positive;

at least 84%, 85%, 90%, 95%, or 99% of the cells in the population are GPA-positive;

54-99%, 55-98%, 60-95%, 65-90%, 70-85%, or 75-80% of the cells in the population are band3-positive;

at least 54%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% of the cells in the population are band3-positive;

96-100%, 97-99%, or about 98% of the cells in the population are alpha4 integrin-positive; or

at least 95%, 96%, 97%, 98%, or 99% of the cells in the population are alpha4 integrin-positive.

59. The method of any of claims 43-58, wherein the mRNA is in vitro transcribed mRNA.

60. The method of any of claims 43-59, wherein at least 80%, 85%, 90%, or 95% of the cells of the population are viable 5 days after the cells are contacted with the mRNA.

61. The method of any of claims 43-60, wherein at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the cells of the population are enucleated 5 days after the cells are contacted with the mRNA.

62. The method of any of claims 43-61, wherein the proportion of cells that are enucleated 5 days after the cells are contacted with the mRNA is at least 50%, 60%, 70%, 80%, 90%, or 95% of the proportion of cells that are enucleated in an otherwise similar population of cells not treated with the ribonuclease inhibitor.

63. The method of any of claims 43-62, wherein the population of cells comprises at least 1 x 10^6, 2 x 10^6, 5 x 10^6, 1 x 10^7, 2 x 10^7, 5 x 10^7, or 1 x 10^8 cells at the time the cells are contacted with the mRNA.

64. The method of any of claims 43-63, wherein the population of cells expands by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% within 5 days after the cells are contacted with the mRNA.

65. The method of any of claims 43-64, wherein at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population express the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA.

66. The method of any of claims 43-65, wherein at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population comprise at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA.

67. The method of any of claims 43-66, wherein the population of cells comprises at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% more, or at least 2-fold, 3-fold, 4-fold, or 5-fold more of the exogenous protein than an otherwise similar population of cells not treated with the ribonuclease inhibitor.

68. A reaction mixture comprising: i) an erythroid cell, ii) an mRNA comprising an exogenous protein and iii) a ribonuclease inhibitor.

69. The reaction mixture of claim 68, wherein the mRNA is inside the erythroid cell.

70. The reaction mixture of claim 68 or 69, which comprises a plurality of erythroid cells.

71. A method of assaying a reaction mixture comprising enucleated erythroid cells that comprise an exogenous protein for a ribonuclease inhibitor, comprising:

providing a reaction mixture comprising enucleated erythroid cells that comprise an exogenous protein,

assaying for the presence or level of a ribonuclease inhibitor, e.g., by ELISA, Western blot, or mass spectrometry, e.g., in an aliquot of the reaction mixture.

72. The method of claim 71, further comprising comparing the level of ribonuclease inhibitor to a reference value.

73. The method of claim 72, further comprising responsive to the comparison, one or more of:

classifying the population, e.g., as meeting a requirement or not meeting a requirement, e.g., wherein the requirement is met when the level of ribonuclease inhibitor is below the reference value,

classifying the population as suitable or not suitable for a subsequent processing step, e.g., when the population is suitable for a subsequent purification step when the level of ribonuclease inhibitor is above the reference value,

classifying the population as suitable or not suitable for use as a therapeutic, or

formulating or packaging the population, or an aliquot thereof, for therapeutic use, e.g., when the level of ribonuclease inhibitor is below the reference value.

74. The reaction mixture or method of any of claims 43-73, wherein the ribonuclease inhibitor is RNAsin Plus, Protector RNAse Inhibitor , or Ribonuclease Inhibitor Huma.

75. A method of making an erythroid cell comprising an mRNA that encodes an exogenous protein, comprising:

providing a reaction mixture comprising an erythroid cell and an mRNA encoding the exogenous protein, under conditions which inhibit protein degradation, e.g., by inclusion in the reaction mixture a proteasome inhibitor, and

maintaining the reaction mixture under conditions that allow uptake of the mRNA by the erythroid cell,

thereby making an erythroid cell comprising an mRNA encoding an exogenous protein.

76. The method of claim 75, comprising providing a population of erythroid cells and contacting the population with the mRNA encoding the exogenous protein.

77. The method of claim 75 or 76, wherein a plurality of erythroid cells of the population each takes up an mRNA encoding the exogenous protein.

78. The method of any of claims 75-77, wherein the cell or plurality of cells express the exogenous protein.

79. The method of any of claims 75-78, wherein the cell or plurality of cells comprise the exogenous protein.

80. The method of any of claims 75-79, which further comprises electroporating the cell or population of cells.

81. The method of any of claims 75-80, which further comprises contacting a population of erythroid cells with a proteasome inhibitor.

82. The method of any of claims 75-81, which comprises contacting the population of cells with the proteasome inhibitor before, during, or after contacting the cells with the mRNA, e.g., 0.5-2 days before or after contacting the cells with the mRNA.

83. The method of any of claims 75-82, which comprises contacting the cells with the proteasome inhibitor at day 4, 5, or 6 of maturation phase.

84. The method of any of claims 75-83, wherein the cell is in maturation phase.

85. The method of any of claims 75-84, which comprises contacting the cells with the proteasome inhibitor at a time when the cells comprise one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or more) of the following properties:

i.a) 2-40%, 3-33%, 5-30%, 10-25%, or 15-20% of the cells in the population are enucleated;

i.b) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 2%, 3%, 4%, or 5% of the cells in the population are enucleated;

i.c) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 6%, 10%, 15%, 20%, or 25% of the cells in the population are enucleated;

i.d) greater than 0%, 0.1%, 0.2%, or 0.5%, but less than 30%, 35%, 40%, 45%, or 50% of the cells in the population are enucleated;

i.e) no more than 1%, 2%, 3%, 5%, 10%, 15%, or 20% of the cells in the population are enucleated;

i.f) no more than 25%, 30%, 35%, 40%, 45%, or 50% of the cells in the population are enucleated;

i.g) the population of cells has reached 6-70%, 10-60%, 20-50%, or 30-40% of maximal enucleation;

i.h) the population of cells has reached no more than 1%, 2%, 3%, 5%, 10%, 15%, or 20% of maximal enucleation;

i.i) the population of cells has reached no more than 25%, 30%, 35%, 40%, 45%, 50%, or 60% of maximal enucleation;

ii.a) the population of cells is fewer than 3, 2, or 1 population doubling from a plateau in cell division;

ii.b) the population of cells is capable of fewer than 3, 2, or 1 population doubling;

ii.c) the population will increase by no more than 1.5, 2, or 3 fold before the population reaches an enucleation level of at least 70% of cells in the population;

iii.a) at least 80%, 85%, 90%, 95%, or 99% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.b) at least 50%, 60%, 70%, 75%, or 79% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.c) 30-90%, 40-90%, 50-90%, 60-90%, or 70-90% of the cells in the population are normoblasts (e.g., polychromatic or orthochromatic normoblasts);

iii.d) at least 80%, 85%, 90%, 95%, or 99% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast);

iii.e) at least 50%, 60%, 70%, 75%, or 79% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast); or

iii.f) 30-90%, 40-90%, 50-90%, 60-90%, or 70-90% of the cells in the population exhibit the morphology of a normoblast (e.g., a polychromatic or orthochromatic normoblast).

86. The method of claim 85, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i and a property from ii.

87. The method of claim 85, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i and a property from iii.

88. The method of claim 85, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from ii and a property from iii.

89. The method of claim 85, wherein the population of erythroid cells or the population of differentiating erythroid cells is a population of erythroid cells comprising a property from i, a property from ii, and a property from iii.

90. The method of any of claims 75-89, which comprises contacting the cells with the proteasome inhibitor at a time when (e.g., by a flow cytometry assay, e.g., a flow cytometry assay of Example 10) the cells comprise one or more (e.g., 2, 3, 4, 5, or more) of the following properties:

84-99%, 85-95%, or about 90% of the cells in the population are GPA-positive;

at least 84%, 85%, 90%, 95%, or 99% of the cells in the population are GPA-positive;

54-99%, 55-98%, 60-95%, 65-90%, 70-85%, or 75-80% of the cells in the population are band3-positive;

at least 54%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% of the cells in the population are band3-positive;

96-100%, 97-99%, or about 98% of the cells in the population are alpha4 integrin-positive; or

at least 95%, 96%, 97%, 98%, or 99% of the cells in the population are alpha4 integrin-positive.

91. The method of any of claims 75-90, wherein the mRNA is in vitro transcribed mRNA.

92. The method of any of claims 75-91, wherein at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population are viable 5 days after the cells are contacted with the mRNA.

93. The method of any of claims 75-92, wherein at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the cells of the population are enucleated 5 days after the cells are contacted with the mRNA.

94. The method of any of claims 75-93, wherein the proportion of cells that are enucleated 5 days after the cells are contacted with the mRNA is at least 50%, 60%, 70%, 80%, 90%, or 95%

of the proportion of cells that are enucleated in an otherwise similar population of cells not treated with the proteasome inhibitor.

95. The method of any of claims 75-94, wherein the population of cells comprises at least 1 x 10^6, 2 x 10^6, 5 x 10^6, 1 x 10^7, 2 x 10^7, 5 x 10^7, or 1 x 10^8 cells at the time the cells are contacted with the mRNA.

96. The method of any of claims 75-95, wherein the population of cells expands by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% within 5 days after the cells are contacted with the mRNA.

97. The method of any of claims 75-96, wherein at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population express the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA.

98. The method of any of claims 75-97, wherein at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population comprise at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the exogenous protein, e.g., 5 days after the cells are contacted with the mRNA.

99. The method of any of claims 75-98, wherein the population of cells comprises at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% more, or at least 2-fold, 3-fold, 4-fold, or 5-fold more of the exogenous protein than an otherwise similar population of cells not treated with the proteasome inhibitor.

100. A reaction mixture comprising: i) an erythroid cell, ii) an mRNA comprising an exogenous protein and iii) a proteasome inhibitor.

101. The reaction mixture of claim 100, wherein the mRNA is inside the erythroid cell.

102. The reaction mixture of claim 100 or 101, which comprises a plurality of erythroid cells.

103. A method of assaying a reaction mixture comprising enucleated erythroid cells that comprise an exogenous protein for a proteasome inhibitor, comprising:

providing a reaction mixture comprising enucleated erythroid cells that comprise an exogenous protein,

assaying for the presence or level of a proteasome inhibitor, e.g., by ELISA, Western blot, or mass spectrometry, e.g., in an aliquot of the reaction mixture.

104. The method of claim 103, further comprising comparing the level of proteasome inhibitor to a reference value.

105. The method of claim 104, further comprising, responsive to the comparison, one or more of:

classifying the population, e.g., as meeting a requirement or not meeting a requirement, e.g., wherein the requirement is met when the level of proteasome inhibitor is below the reference value,

classifying the population as suitable or not suitable for a subsequent processing step, e.g., when the population is suitable for a subsequent purification step when the level of proteasome inhibitor is above the reference value,

classifying the population as suitable or not suitable for use as a therapeutic, or

formulating or packaging the population, or an aliquot thereof, for therapeutic use, e.g., when the level of proteasome inhibitor is below the reference value.

106. The reaction mixture or method of any of claims 75-105, wherein the proteasome inhibitor is a 20S proteasome inhibitor, e.g., MG-132 or carfilzomib, or a 26S proteasome inhibitor, e.g., bortezomib.

107. A method of making an erythroid cell comprising an mRNA encoding a first exogenous protein and a second exogenous protein, comprising:

a) providing an erythroid cell, e.g., in maturation phase, and

b) contacting the erythroid cell with an mRNA encoding the first exogenous protein and a second mRNA encoding the second exogenous protein, under conditions that allow uptake of the first mRNA and second mRNA by the erythroid cell,

thereby making an erythroid cell comprising the first mRNA and the second mRNA.

108. The method of claim 107, wherein the erythroid cell comprises at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the first exogenous protein and the second exogenous protein, e.g., 5 days after the contacting with the mRNA.

109. A method of producing a population of erythroid cells expressing a first exogenous protein and a second exogenous protein, comprising:

a) providing a population of erythroid cells, e.g., in maturation phase, and

b) contacting the population of erythroid cells with a first mRNA encoding a first protein and a second mRNA encoding a second protein,

thereby making an erythroid cell comprising an mRNA encoding an exogenous protein

wherein at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of cells in the population comprise both of the first mRNA and the second mRNA.

110. The method of claim 109, wherein the population of erythroid cells comprises an average of at least 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, or 100,000 copies of the first exogenous protein and the second exogenous protein per cell, e.g., 5 days after the contacting with the mRNA.

111. The method of any of claims 107-110, wherein the contacting comprises performing electroporation.

112. The method of any of claims 109-111, wherein the population of cells comprise the first exogenous protein and the second exogenous protein in at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of cells for at least 5 days after the cells were contacted with the first and second mRNAs.

113. A population of erythroid cells wherein at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of cells in the population express a first exogenous protein and a second exogenous protein, wherein the population was not made by contacting the cells with DNA encoding the first or second exogenous protein.

114. A method of producing a plurality of erythroid cells comprising a predetermined number of copies of an exogenous protein per cell, comprising contacting the population with a predetermined amount of mRNA encoding the exogenous protein, thereby making the erythroid cell comprising the predetermined amount of the exogenous protein.

115. The method of claim 114, further comprising evaluating one or more of the plurality of erythroid cells (e.g., enucleated erythroid cells) to determine the amount of the exogenous protein.

116. A method of evaluating the amount of an exogenous protein in a sample of erythroid cells, e.g., enucleated erythroid cells comprising:

providing a plurality of erythroid cells comprising a predetermined number of copies of an exogenous protein per cell, which was made by contacting the population with a predetermined amount of mRNA encoding the exogenous protein, and

determining the amount of the exogenous protein in the plurality of erythroid cells.

117. The method of claim any of claims 114-116, wherein:

contacting the cell population with 0.6 ±20% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 1,000,000 ±20% copies of the exogenous protein per cell,

contacting the cell population with 0.4 ±20% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 870,000 ±20% copies of the exogenous protein per cell,

contacting the cell population with 0.2 ±20% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 610,000 ±20% copies of the exogenous protein per cell,

contacting the cell population with 0.1±20% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 270,000±20% copies of the exogenous protein per cell,

contacting the cell population with 0.05±20% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 100,000 ±20% copies of the exogenous protein per cell, or

contacting the cell population with 0.025 ±20% ug of mRNA per 5E6 cells in the population yields a population of cells expressing 43,000 ±20% copies of the exogenous protein per cell.

118. The method of any of claims 114-117, wherein at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% of the cells of the population express the exogenous protein 1 day after the cells are contacted with the exogenous protein.

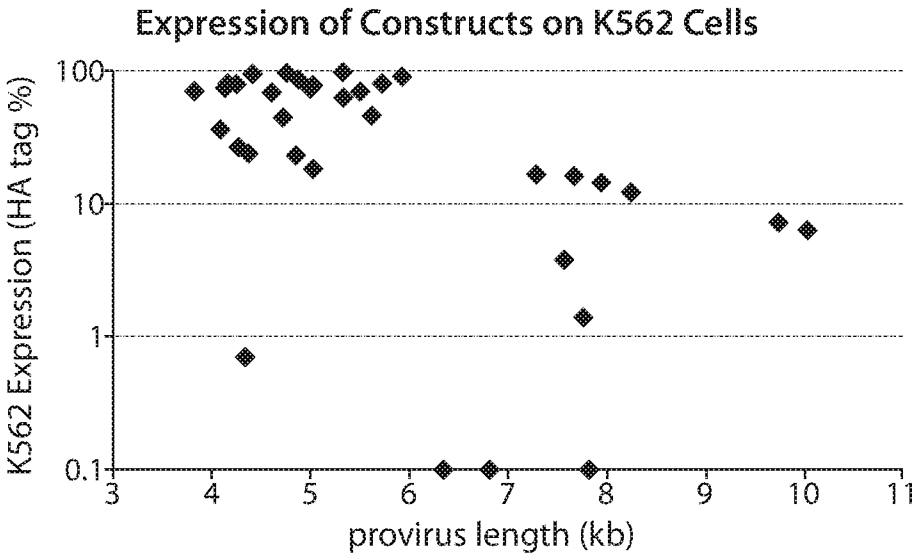
Expression of Constructs on K562 Cells
K562 Expression (HA tag %)
100
10
1
0.1
3
4
5
6
7
8
9
10
11
provirus length (kb)

Fig. 1

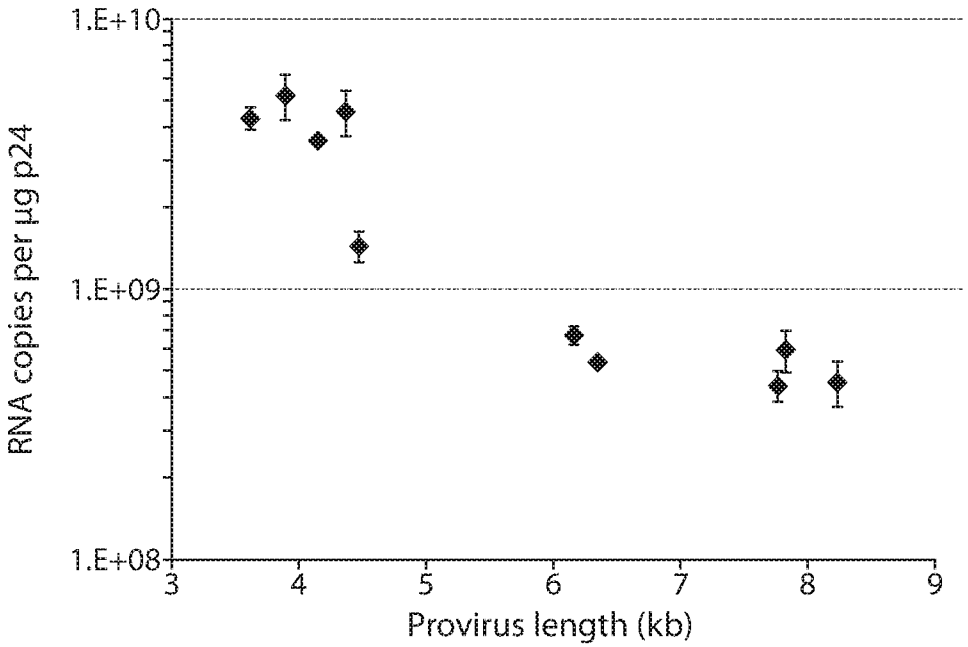
1.E+10
1.E+09
1.E+08
RNA copies per µg p24
3
4
5
6
7
8
9
Provirus length (kb)

Fig. 2

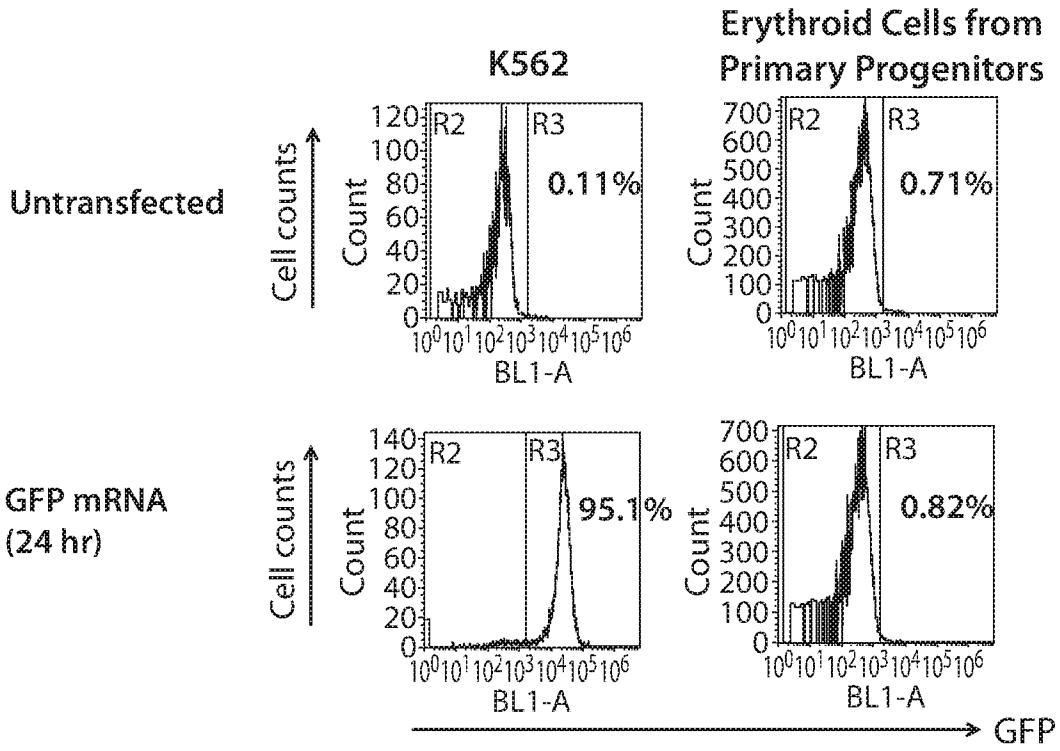
K562
Erythroid Cells from Primary Progenitors
Untransfected
GFP mRNA (24 hr)
Cell counts
Count
R2
R3
0.11%
0.71%
95.1%
0.82%
BL1-A
GFP

Fig. 3

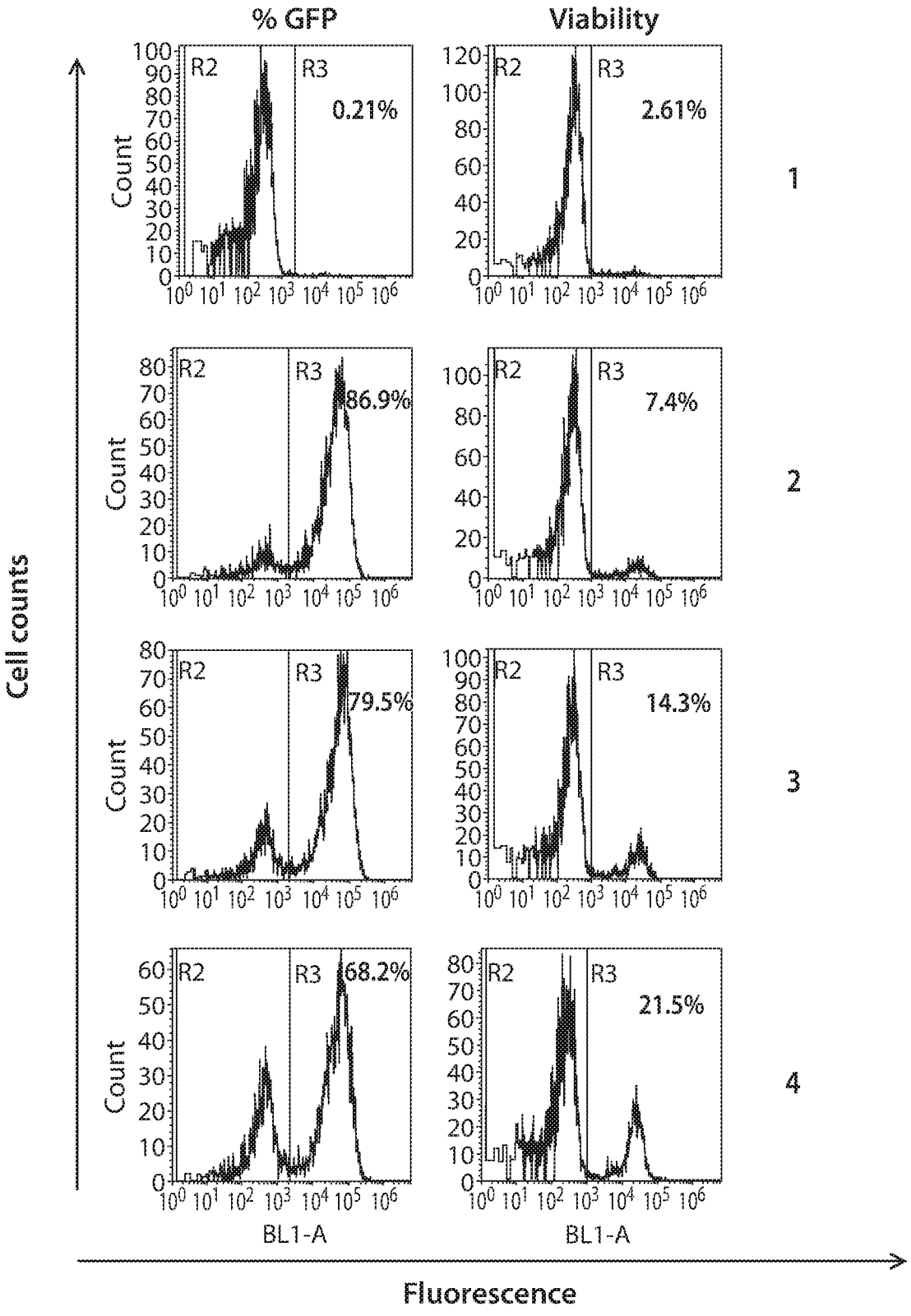
% GFP
Viability
R2
R3
0.21%
2.61%
86.9%
7.4%
79.5%
14.3%
68.2%
21.5%
Count
Cell counts
BL1-A
Fluorescence
1
2
3
4

Fig. 4A

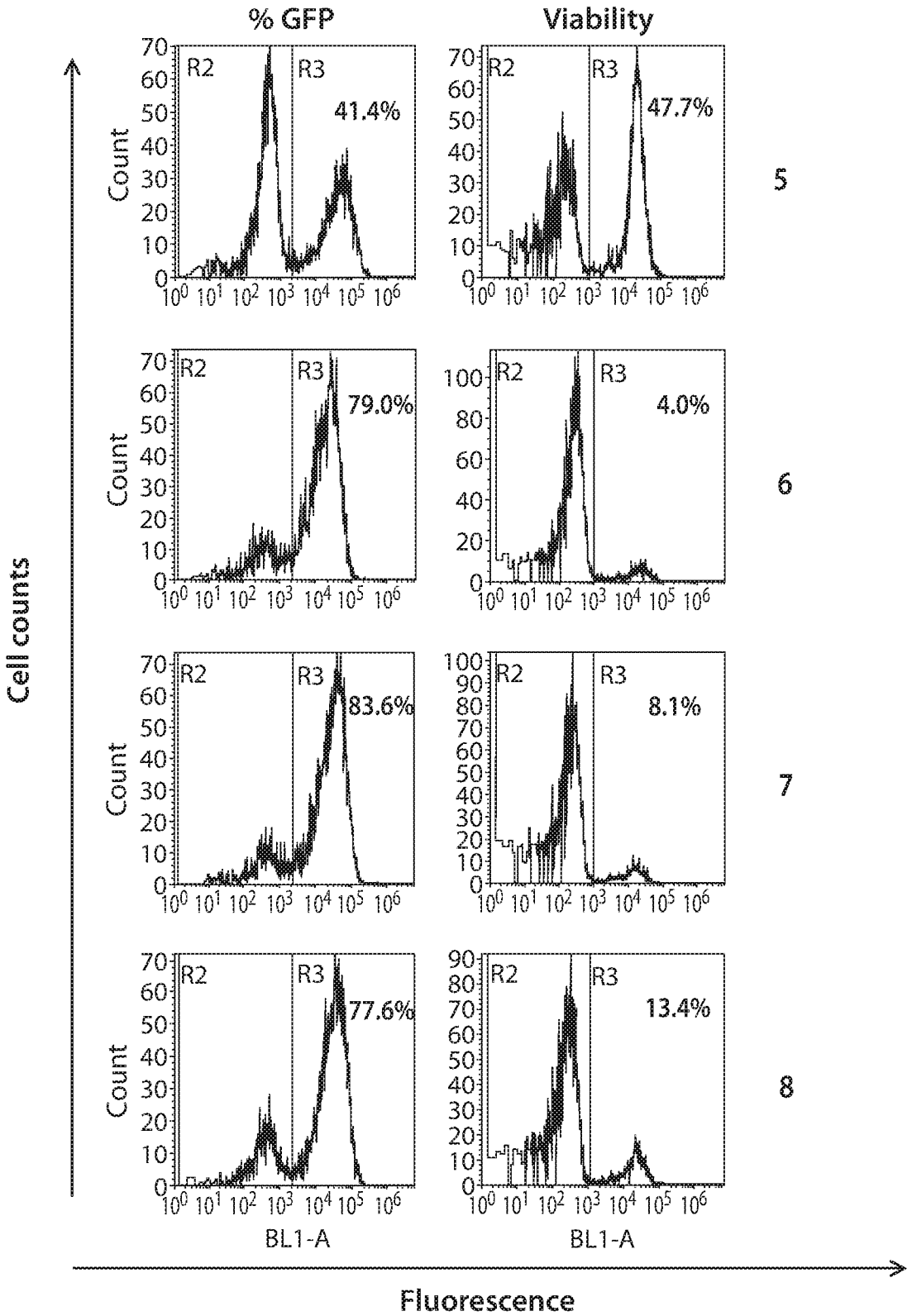
% GFP
Viability
R2
R3
41.4%
47.7%
79.0%
4.0%
83.6%
8.1%
77.6%
13.4%
Count
5
6
7
8
Cell counts
BL1-A
BL1-A
Fluorescence

Fig. 4B

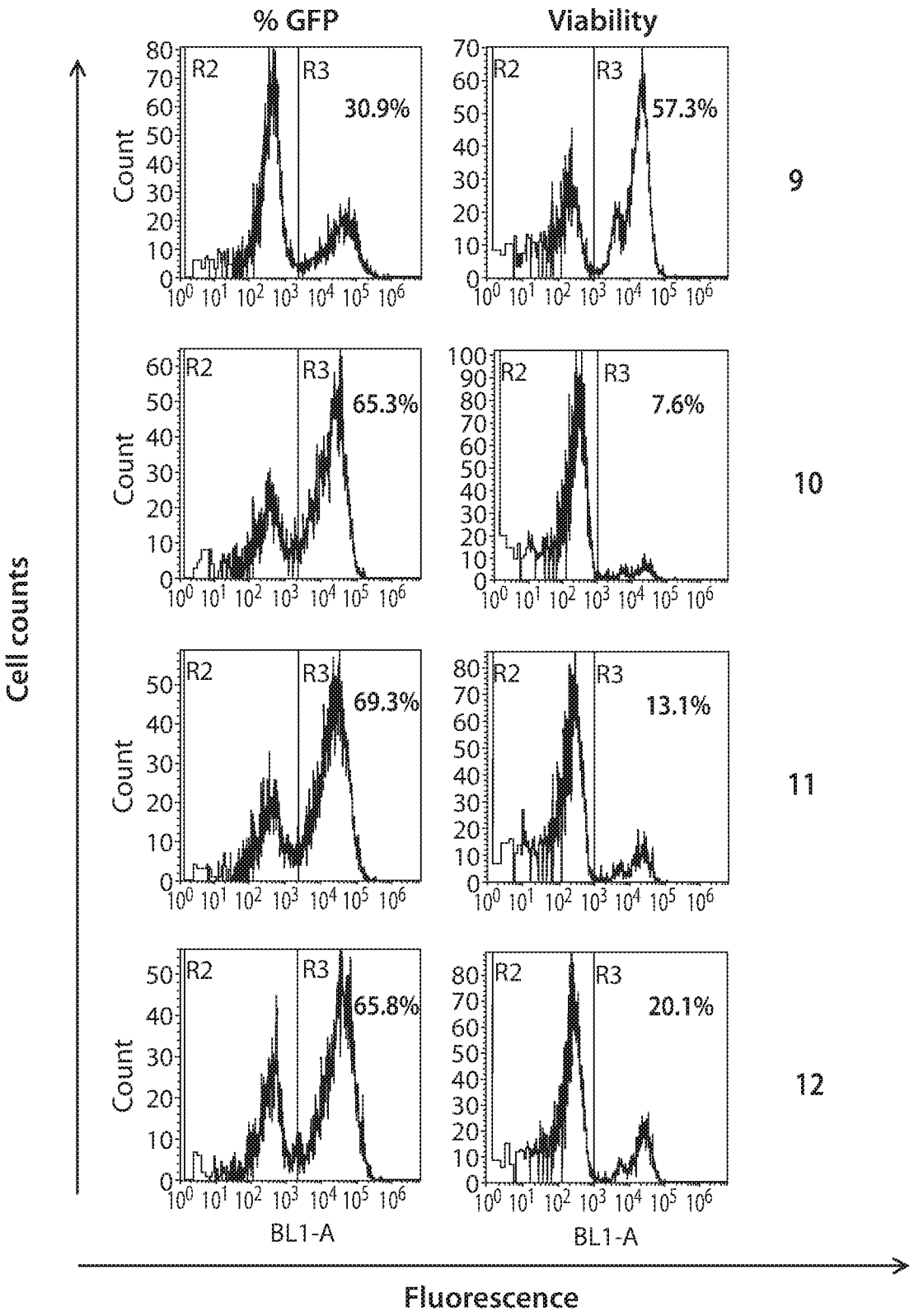
% GFP
Viability
R2
R3
30.9%
57.3%
65.3%
7.6%
69.3%
13.1%
65.8%
20.1%
Count
BL1-A
9
10
11
12
Cell counts
Fluorescence

Fig. 4C

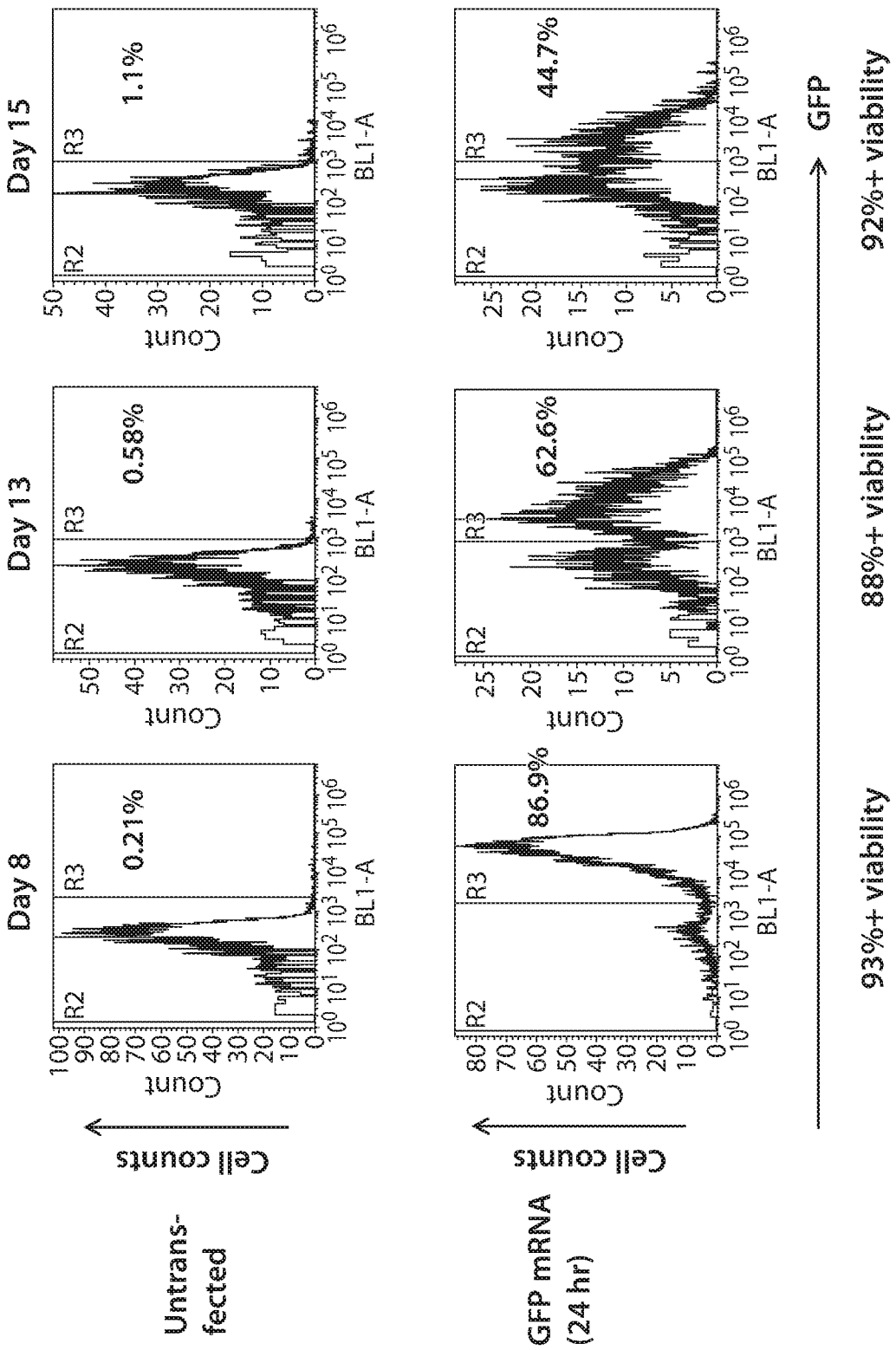
Day 8
Day 13
Day 15
Untrans-fected
GFP mRNA (24 hr)
Cell counts
Count
BL1-A
R2
R3
0.21%
0.58%
1.1%
86.9%
62.6%
44.7%
93%+ viability
88%+ viability
92%+ viability
GFP

Fig. 5

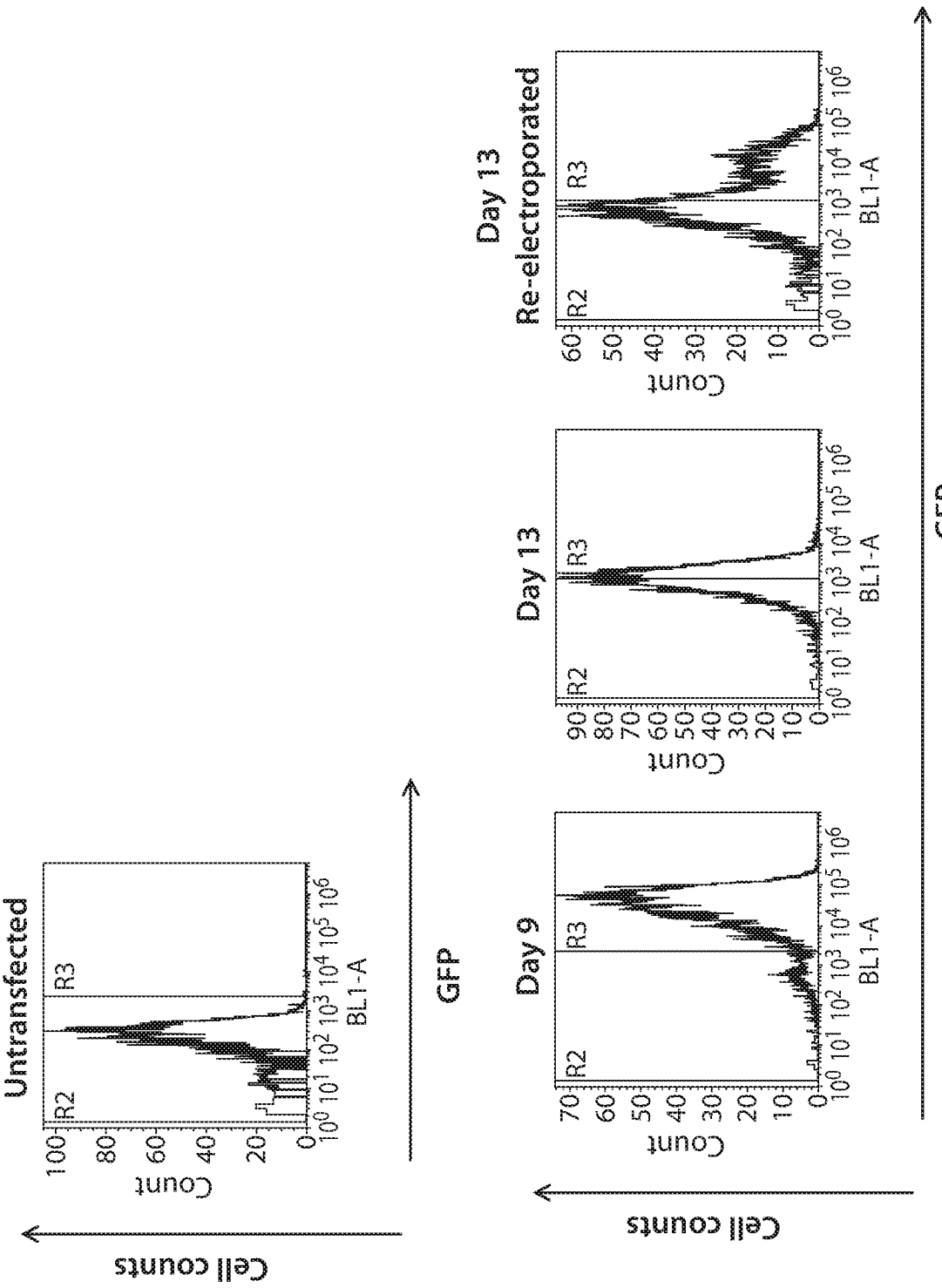
Untransfected
Day 9
Day 13
Day 13
Re-electroporated
R2
R3
Count
BL1-A
Cell counts
GFP

Fig. 6

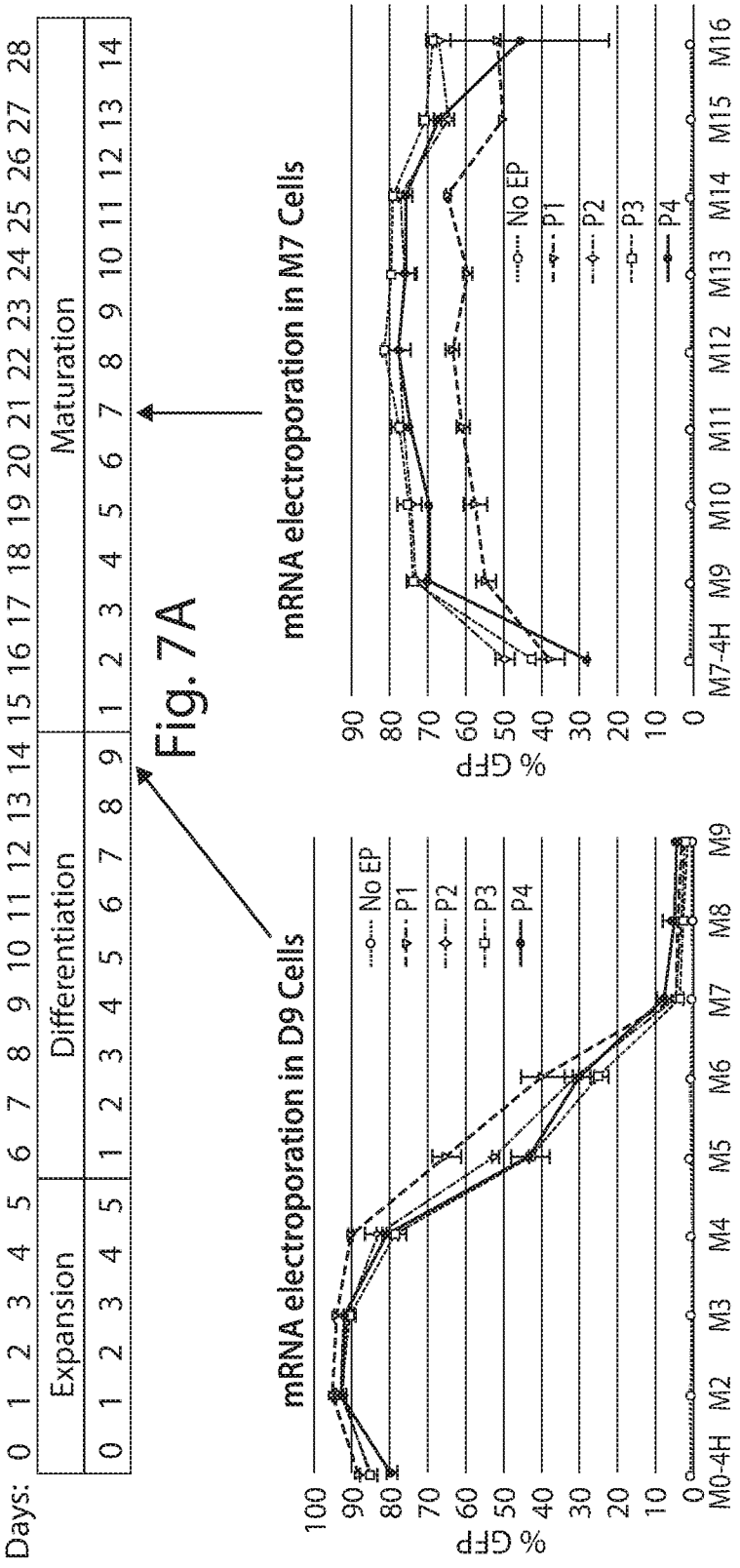

Fig. 7A

Fig. 7B

Fig. 7C

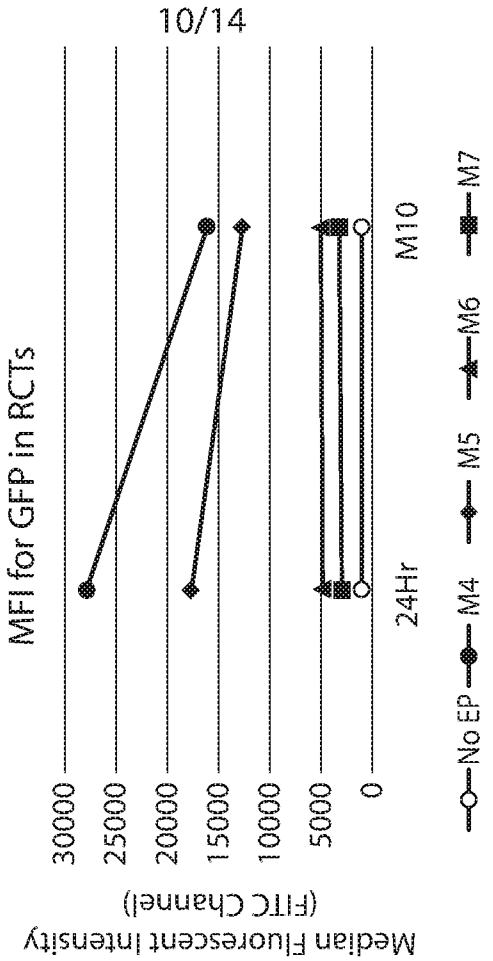
MFI for GFP in RCTs
Median Fluorescent Intensity
(FITC Channel)
30000
25000
20000
15000
10000
5000
0
24Hr
M10
No EP
M4
M5
M6
M7

Fig. 8B

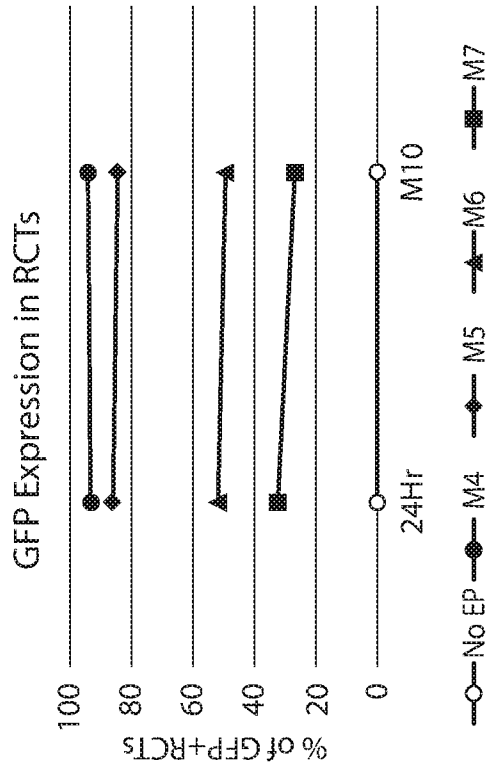
GFP Expression in RCTs
% of GFP+RCTs
100
80
60
40
20
0
24Hr
M10
No EP
M4
M5
M6
M7

Fig. 8A

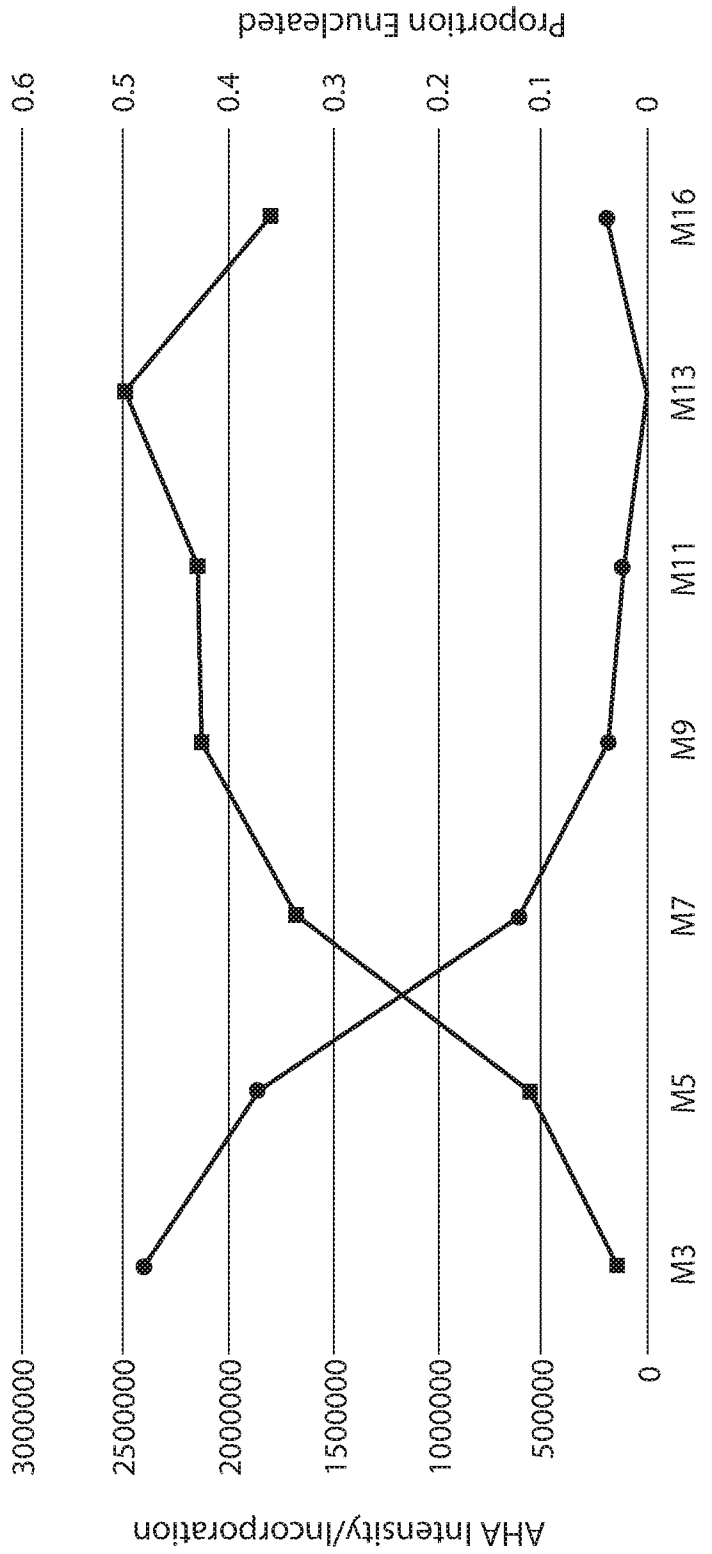
AHA Intensity/Incorporation
3000000
2500000
2000000
1500000
1000000
500000
0
Proportion Enucleated
0.6
0.5
0.4
0.3
0.2
0.1
0
M3
M5
M7
M9
M11
M13
M16
Maturation Day

Fig. 9

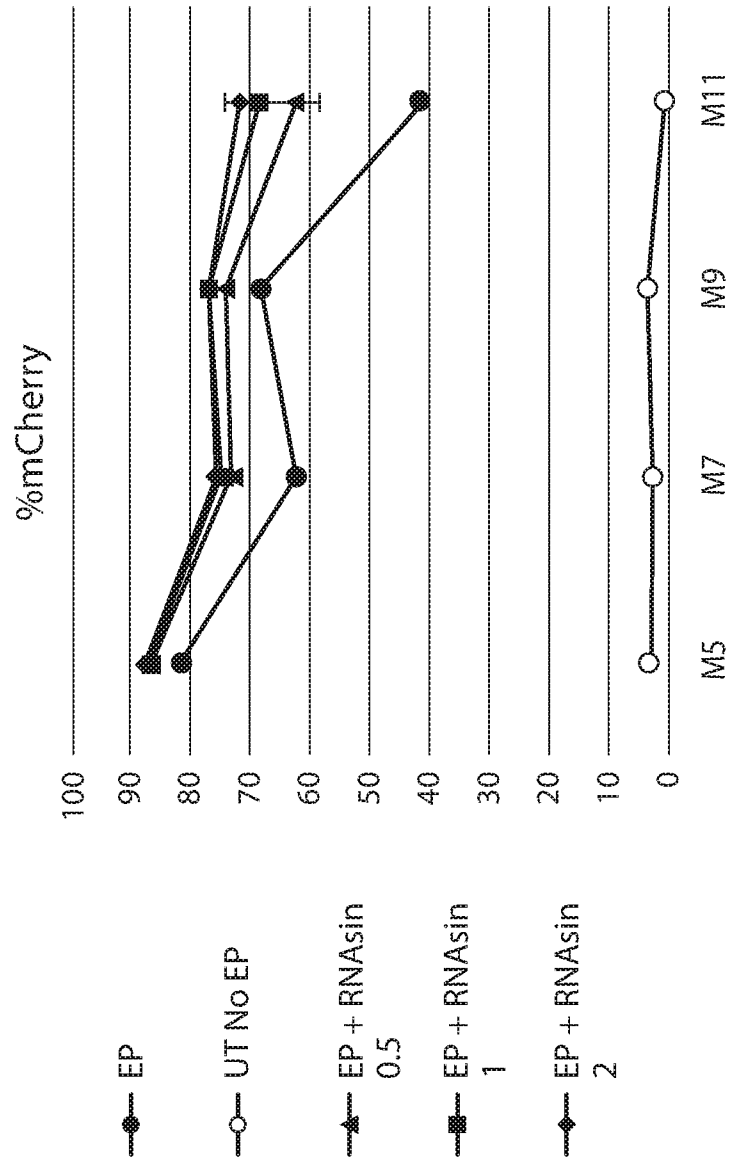
%mCherry
100
90
80
70
60
50
40
30
20
10
0
M5
M7
M9
M11
EP
UT No EP
EP + RNAsin 0.5
EP + RNAsin 1
EP + RNAsin 2

Fig. 10

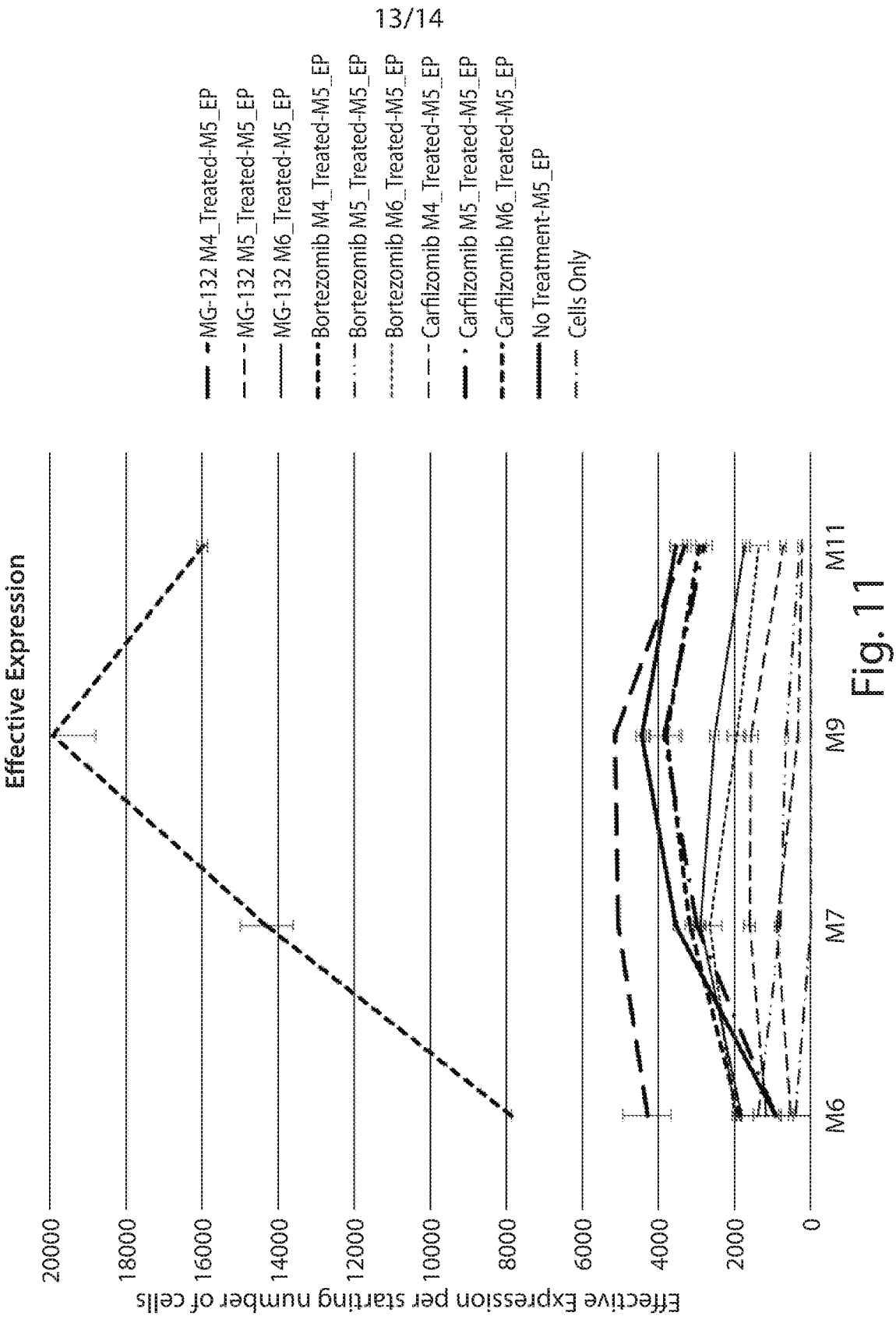
Effective Expression
Effective Expression per starting number of cells
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
M6
M7
M9
M11
MG-132 M4_Treated-M5_EP
MG-132 M5_Treated-M5_EP
MG-132 M6_Treated-M5_EP
Bortezomib M4_Treated-M5_EP
Bortezomib M5_Treated-M5_EP
Bortezomib M6_Treated-M5_EP
Carfilzomib M4_Treated-M5_EP
Carfilzomib M5_Treated-M5_EP
Carfilzomib M6_Treated-M5_EP
No Treatment-M5_EP
Cells Only

Fig. 11

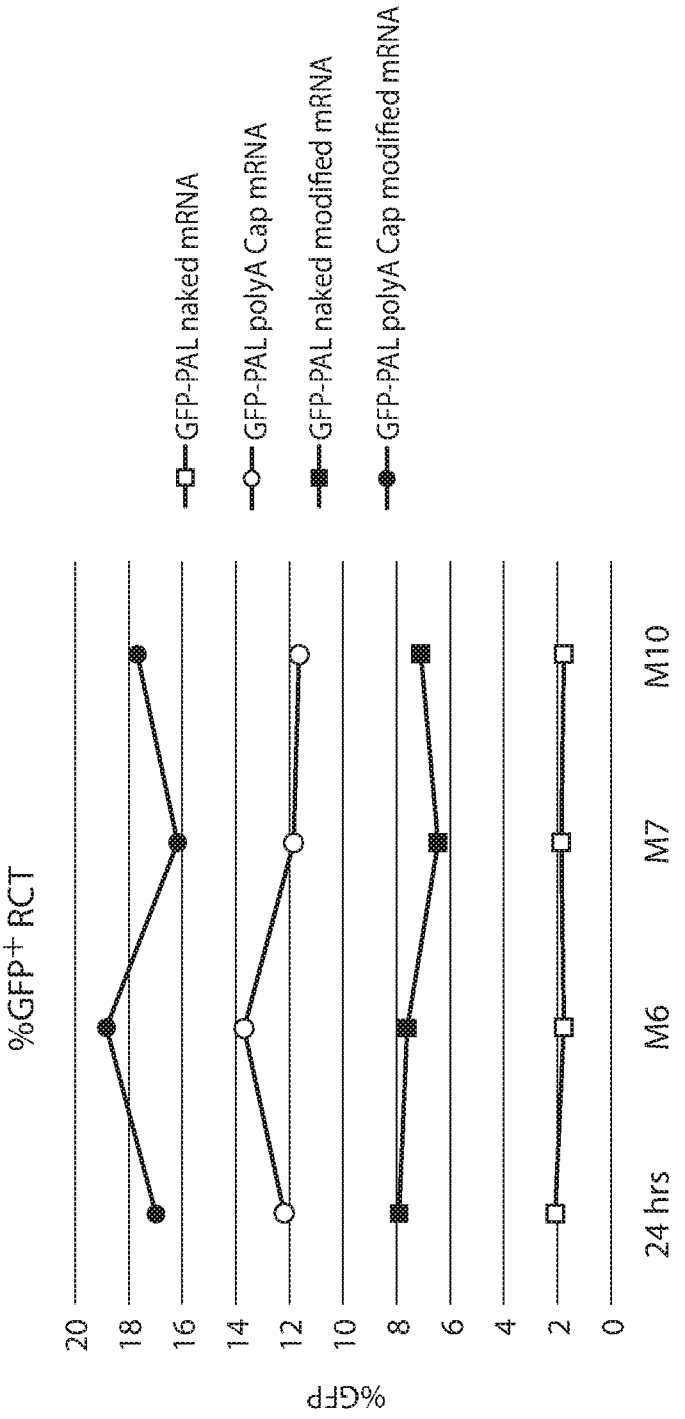
GFP-PAL naked mRNA
GFP-PAL polyA Cap mRNA
GFP-PAL naked modified mRNA
GFP-PAL polyA Cap modified mRNA
%GFP+ RCT
%GFP
20
18
16
14
12
10
8
6
4
2
0
24 hrs
M6
M7
M10

Fig. 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/041155

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N5/078
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CAB Data, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/073587 A2 (RUBIUS THERAPEUTICS INC [US]) 21 May 2015 (2015-05-21)	1-7
Y	the whole document	107-113

X	WO 2015/153102 A1 (RUBIUS THERAPEUTICS INC [US]) 8 October 2015 (2015-10-08)	1-7
Y	the whole document	107-113

X,P	WO 2016/183482 A1 (RUBIUS THERAPEUTICS INC [US]) 17 November 2016 (2016-11-17)	1-7
Y,P	the whole document	107-113

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 September 2017	01/12/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Hornig, Horst

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/041155

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-7, 107-113

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-7, 107-113

A method of making an erythroid cell comprising an mRNA encoding an exogenous protein, comprising: a) providing an erythroid cell in maturation phase, and b) contacting the erythroid cell with an mRNA encoding the exogenous protein, under conditions that allow uptake of the mRNA by the erythroid cell, thereby making an erythroid cell comprising an mRNA encoding an exogenous protein; A method of making an erythroid cell comprising an mRNA encoding a first exogenous protein and a second exogenous protein, comprising: a) providing an erythroid cell, e.g., in maturation phase, and b) contacting the erythroid cell with an mRNA encoding the first exogenous protein and a second mRNA encoding the second exogenous protein, under conditions that allow uptake of the first mRNA and second mRNA by the erythroid cell, thereby making an erythroid cell comprising the first mRNA and the second mRNA; A population of erythroid cells wherein at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of cells in the population express a first exogenous protein and a second exogenous protein, wherein the population was not made by contacting the cells with DNA encoding the first or second exogenous protein;

---

2. claims: 8-29

A method of manufacturing a population of reticulocytes that express an exogenous protein, the method comprising: (e) providing a population of erythroid precursor cells (e.g., CD34+ cells); (f) culturing the population of erythroid precursor cells under differentiating conditions to provide a population of differentiating erythroid cells;

---

3. claims: 30, 32, 34, 36, 38, 40-42

An erythroid cell, e.g., an enucleated erythroid cell, comprising: an exogenous mRNA comprising a coding region operatively linked to a heterologous untranslated region (UTR), wherein the heterologous UTR comprises a regulatory element; A method of producing an erythroid cell, e.g., enucleated erythroid cell, comprising: a) contacting an erythroid cell, e.g., a nucleated erythroid cell, with an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element, (e.g., isolated RNA or in vitro transcribed RNA), and b) maintaining the contacted erythroid cell under conditions suitable for uptake of the exogenous mRNA, thereby producing the erythroid cell, e.g., an enucleated erythroid cell; A method of providing a subject with an exogenous protein, providing a subject with an enucleated erythroid cell which

can produce an exogenous protein, or treating a subject, comprising administering to the subject: comprising an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element, (e.g., isolated RNA or in vitro transcribed RNA); A method of evaluating an erythroid cell, e.g., enucleated erythroid cell (or a batch of such cells) comprising: a) providing an erythroid cell, e.g., a nucleated erythroid cell, comprising an exogenous mRNA comprising a coding region operatively linked to a heterologous UTR comprising a regulatory element (or a batch of such cells);

---

4. claims: 31, 33, 37

An erythroid cell, e.g., an enucleated erythroid cell, comprising an exogenous mRNA that comprises one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof; A method of producing an erythroid cell, e.g., enucleated erythroid cell, comprising: a) contacting an erythroid cell, e.g., a nucleated erythroid cell, with an exogenous mRNA comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof; A method of providing a subject with an exogenous protein, providing a subject with an enucleated erythroid cell which can produce an exogenous protein, or treating a subject, comprising administering to the subject: an erythroid cell, e.g., a nucleated erythroid cell, comprising an exogenous mRNA comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof;

---

5. claims: 35, 39

A method of producing an exogenous protein in an enucleated erythroid cell: a) providing an erythroid cell, e.g., a nucleated erythroid cell, comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination thereof, and b) culturing the erythroid cell under conditions suitable for production of the exogenous protein, thereby producing the exogenous protein [NO mRNA]; A method of evaluating an erythroid cell, e.g., enucleated erythroid cell (or a batch of such cells) comprising: a) providing an erythroid cell, e.g., a nucleated erythroid cell, comprising one or more chemically modified nucleotides of Table 1, one or more chemical backbone modifications of Table 2, one or more chemically modified caps of Table 3, or any combination

thereof, and b) evaluating the erythroid cell, e.g., the nucleated erythroid cell (or batch of such cells) for a preselected parameter, thereby evaluating the erythroid cell, e.g., enucleated erythroid cell (or a batch of such cells) [NO mRNA]

---

6. claims: 43-74

A method of making an erythroid cell comprising an mRNA that encodes an exogenous protein, comprising: providing a reaction mixture comprising an erythroid cell and an mRNA encoding the exogenous protein, under conditions which inhibit degradation of mRNA, e.g., by inclusion in the reaction mixture a ribonuclease inhibitor, and maintaining the reaction mixture under conditions that allow uptake of the mRNA by the erythroid cell, thereby making an erythroid cell comprising an mRNA encoding an exogenous protein; A reaction mixture comprising: i) an erythroid cell, ii) an mRNA comprising an exogenous protein and iii) a ribonuclease inhibitor;

---

7. claims: 75-106

A method of making an erythroid cell comprising an mRNA that encodes an exogenous protein, comprising: providing a reaction mixture comprising an erythroid cell and an mRNA encoding the exogenous protein, under conditions which inhibit protein degradation, e.g., by inclusion in the reaction mixture a proteasome inhibitor, and maintaining the reaction mixture under conditions that allow uptake of the mRNA by the erythroid cell, thereby making an erythroid cell comprising an mRNA encoding an exogenous protein; A reaction mixture comprising: i) an erythroid cell, ii) an mRNA comprising an exogenous protein and iii) a proteasome inhibitor;

---

8. claims: 114-118

A method of producing a plurality of erythroid cells comprising a predetermined number of copies of an exogenous protein per cell, comprising contacting the population with a predetermined amount of mRNA encoding the exogenous protein, thereby making the erythroid cell comprising the predetermined amount of the exogenous protein; A method of evaluating the amount of an exogenous protein in a sample of erythroid cells, e.g., enucleated erythroid cells comprising: providing a plurality of erythroid cells comprising a predetermined number of copies of an exogenous protein per cell, which was made by contacting the population with a predetermined amount of mRNA encoding the exogenous protein, and determining the amount of the exogenous protein in the plurality of erythroid cells.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/041155

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2015073587 A2	21-05-2015	AU 2014348683 A1	09-06-2016
		CA 2930665 A1	21-05-2015
		EP 3071515 A2	28-09-2016
		JP 2017504568 A	09-02-2017
		US 2015182588 A1	02-07-2015
		US 2015306212 A1	29-10-2015
		US 2016257932 A1	08-09-2016
		WO 2015073587 A2	21-05-2015
WO 2015153102 A1	08-10-2015	AU 2015241422 A1	27-10-2016
		CA 2944492 A1	08-10-2015
		CN 106456744 A	22-02-2017
		EP 3125927 A1	08-02-2017
		JP 2017517485 A	29-06-2017
		KR 20170005801 A	16-01-2017
		US 2017020926 A1	26-01-2017
		WO 2015153102 A1	08-10-2015
WO 2016183482 A1	17-11-2016	NONE	