

human mammalian cells can give rise to non-human glycosylation patterns in the recombinant product. Moreover, mammalian cells, including CHO cells, express many proteins that are not essential for cell growth, survival, and/or productivity. Expression of these mammalian cell proteins, however, consumes considerable cellular energy and DNA/protein building blocks. Reducing or eliminating the expression of such proteins can render cell growth more efficient. In addition, in contexts where the cell is used for production of a recombinant product of interest (e.g., a recombinant protein), some of these endogenous proteins can co-purify with the recombinant product of interest, leading to increased costs associated with additional purification process improvements and/or decreased shelf-life of the resulting product. For example, certain residual mammalian cell proteins that co-purify with the product of interest can degrade polysorbate used as a surfactant in the final drug product, and lead to particle formation (Dixit et al., J Pharm Sci, 2016, Volume 105, Issue 5, Pages 1657–1666). Likewise, expression of endogenous retrovirus-like particles (RVLPs) by mammalian cells are undesirable, and considerable burden is placed on downstream processing to demonstrate adequate removal of RVLPs in biotherapeutic manufacturing processes.

Accordingly, there is a need in the art for more efficient methods, mammalian cells, and compositions for producing a recombinant product of interest (e.g., a recombinant protein, a recombinant viral particle, or recombinant viral vector), where the modified mammalian cells expressing the recombinant product of interest exhibit improved attributes relevant to mammalian cell viability, expression, and product quality, as well as facilitating downstream purification of the product of interest. Such improved mammalian cells can be achieved by applying carefully-selected modifications to the genome of the mammalian host cells (i.e., cell line engineering).

3. SUMMARY

In certain examples, the present disclosure is directed to a modified mammalian cell, wherein the cell is modified to reduce or eliminate the expression of one or more endogenous products relative to the expression of the endogenous products in an unmodified cell, wherein the one or more endogenous products: promotes apoptosis of the modified cell during cell culture; promotes clumping and/or aggregation of the modified cell during cell culture; is not essential for the growth, survival, and/or productivity of the modified cell during cell culture; promotes non-human glycosylation patterns in a recombinant protein

product produced by the modified cell during cell culture; can co-purify with the product of interest produced by the modified cell during cell culture; and/or requires removal by purification for product quality and/or safety reasons. In one aspect, the present disclosure is directed to a modified mammalian cell, wherein the cell is modified to reduce or eliminate the expression of one or more endogenous products relative to the expression of the endogenous products in an unmodified cell, wherein the one or more endogenous products: (a) promotes apoptosis of the modified cell during cell culture; (b) promotes clumping and/or aggregation of the modified cell during cell culture; (c) is not essential for the growth, survival, and/or productivity of the modified cell during cell culture; (d) promotes non-human glycosylation patterns in a recombinant protein product produced by the modified cell during cell culture; (e) can co-purify with the product of interest produced by the modified cell during cell culture; (f) promotes branched chain amino acid catabolism; and/or (g) requires removal by purification for product quality and/or safety reasons.

In certain examples, the present disclosure is directed to a modified mammalian cell, wherein the cell is modified to reduce or eliminate the expression of one or more endogenous products relative to the expression of the endogenous products in an unmodified cell, wherein the one or more endogenous products is selected from endogenous virus-like particles such as retrovirus-like particles (RVLPs), e.g., via a reduction or elimination of RVLP group antigen (GAG) expression, and/or one or more of the endogenous protein group consisting of: BCL2 Associated X, Apoptosis Regulator (BAX); BCL2 Antagonist/Killer 1 (BAK); Intercellular Adhesion Molecule 1 (ICAM-1); Protein Kinase R-like ER Kinase (PERK); Sirtuin 1 (SIRT-1); MYC Proto-Oncogene, BHLH Transcription Factor (MYC); Glycoprotein Alpha-Galactosyltransferase 1 (GGTA1); Cytidine Monophosphate-N-Acetylneuraminic Acid Hydroxylase (CMAH); Lipoprotein lipase (LPL); Phospholipase A2 group XV (LPLA2); Palmitoyl-protein thioesterase 1 (PPT1); Branched Chain Keto Acid Dehydrogenase E1 alpha subunit (BCKDHA); Branched Chain Keto Acid Dehydrogenase E1 beta subunit (BCKDHB); and Lipase A (Lysosomal acid lipase/cholesteryl ester hydrolase, Lipase) (LIPA). In one aspect, the present disclosure is directed to a modified mammalian cell, wherein the cell is modified to reduce or eliminate the expression of one or more endogenous products relative to the expression of the endogenous products in an unmodified cell, wherein the one or more endogenous products is selected from the endogenous protein group consisting of: BCL2 Associated X, Apoptosis Regulator (BAX); BCL2 Antagonist/Killer 1 (BAK); Intercellular Adhesion Molecule 1 (ICAM-1); Protein Kinase R-like ER Kinase (PERK); Sirtuin 1 (SIRT-1); MYC Proto-Oncogene, BHLH Transcription Factor (MYC); Glycoprotein Alpha-Galactosyltransferase 1 (GGTA1); Cytidine Monophosphate-N-Acetylneuraminic Acid Hydroxylase (CMAH); Branched Chain Keto Acid Dehydrogenase E1 alpha subunit (BCKDHA); Branched Chain Keto Acid Dehydrogenase E1 beta subunit (BCKDHB); Lipoprotein lipase (LPL); Phospholipase A2 group XV (LPLA2); Palmitoyl-protein thioesterase 1 (PPT1); and Lipase A (Lysosomal acid lipase/cholesteryl ester hydrolase, Lipase) (LIPA).

In certain examples, the present disclosure is directed to a modified cell of wherein the expression of RVLPs is reduced or eliminated, e.g., via a reduction or elimination of RVLP group antigen (GAG) expression.

In certain examples, the present disclosure is directed to a modified cell of wherein the expression of:

- a) BAX; BAK; ICAM-1; GGTA1; CMAH; LPL; LPLA2; and PPT1;
- b) BAX; BAK; ICAM-1; SIRT-1; GGTA1; CMAH; LPL; LPLA2; and PPT1;
- c) BAX; BAK; ICAM-1; SIRT-1; MYC; GGTA1; CMAH; LPL; LPLA2; and PPT1;
- d) BAX; BAK; ICAM-1; SIRT-1; MYC; GGTA1; CMAH; LPL; LPLA2; PPT1; and LIPA;
- e) BAX; BAK; ICAM-1; GGTA1; CMAH; LPLA2; PPT1; and LIPA;
- f) BAX; BAK; ICAM-1; SIRT-1; GGTA1; CMAH; LPLA2; PPT1; and LIPA;
- g) BAX; BAK; ICAM-1; SIRT-1; MYC; GGTA1; CMAH; LPLA2; PPT1; and LIPA;

In certain examples, the present disclosure is directed to a modified cell wherein the expression of:

- a) GAG; BAX; BAK; ICAM-1; GGTA1; CMAH; LPL; LPLA2; and PPT1;
- b) GAG; BAX; BAK; ICAM-1; SIRT-1; GGTA1; CMAH; LPL, LPLA2; and PPT1;
- c) GAG; BAX; BAK; ICAM-1; SIRT-1; MYC; GGTA1; CMAH; LPL; LPLA2; and PPT1;
- d) GAG; BAX; BAK; ICAM-1; SIRT-1; MYC; GGTA1; CMAH; LPL, LPLA2; PPT1; and LIPA;
- e) GAG; BAX; BAK; ICAM-1; GGTA1; CMAH; LPLA2; PPT1; and LIPA;
- f) GAG; BAX; BAK; ICAM-1; SIRT-1; GGTA1; CMAH; LPLA2; PPT1; and LIPA;
- g) GAG; BAX; BAK; ICAM-1; SIRT-1; MYC; GGTA1; CMAH; LPLA2; PPT1; and LIPA;
- h) GAG; BAX; BAK; ICAM-1; LPL; LPLA2; and PPT1;
- i) GAG; BAX; BAK; ICAM-1; SIRT-1; LPL, LPLA2; and PPT1;
- j) GAG; BAX; BAK; ICAM-1; SIRT-1; MYC; LPL; LPLA2; and PPT1;
- k) GAG; BAX; BAK; ICAM-1; SIRT-1; MYC; LPL; LPLA2; PPT1; and LIPA;
- l) GAG; BAX; BAK; ICAM-1; SIRT-1; and MYC;
- m) GAG; BAX; BAK; ICAM-1; PERK; SIRT-1; and MYC;
- n) GAG; BAX; BAK; ICAM-1; PERK; SIRT-1; MYC; GGTA1; CMAH; LPL, LPLA2; PPT1; and LIPA;
- o) GAG; BAX; BAK; LPL; LPLA2; GGTA1; and CMAH;
- p) GAG; BAX; BAK; PERK; LPL; LPLA2; GGTA1; and CMAH;
- q) GAG; BAX; BAK; MYC; LPL; LPLA2; GGTA1; and CMAH;
- r) GAG; BAX; BAK; MYC; PERK; LPL; LPLA2; GGTA1; and CMAH;
- s) GAG; BAX; BAK; LPL; LPLA2; GGTA1; CMAH; and PPT1;
- t) GAG; BAX; BAK; PERK; LPL; LPLA2; GGTA1; CMAH; and PPT1;
- u) GAG; BAX; BAK; MYC; LPL; LPLA2; GGTA1; CMAH; and PPT1;
- v) GAG; BAX; BAK; MYC; PERK; LPL; LPLA2; GGTA1; CMAH; and PPT1;
- w) GAG; BAX; BAK; ICAM-1; and SIRT-1;
- x) GAG; BAX; BAK; and ICAM-1;
- y) BAX; BAK; BCKDHA; ICAM-1; GGTA1; CMAH; LPL; LPLA2; and PPT1;

IIII) BAX; BAK; BCKDHA; BCKDHB; MYC; PERK; LPL; LPLA2; GGTA1; and
 CMAH;
 mmmm) BAX; BAK; BCKDHA; BCKDHB; LPL;
 LPLA2; GGTA1; CMAH; and PPT1;
 5 nnnn) BAX; BAK; BCKDHA; BCKDHB; PERK; LPL; LPLA2; GGTA1;
 CMAH; and PPT1;
 oooo) BAX; BAK; BCKDHA; BCKDHB; MYC; LPL; LPLA2; GGTA1;
 CMAH; and PPT1;
 pppp) BAX; BAK; BCKDHA; BCKDHB; MYC; PERK; LPL; LPLA2;
 10 GGTA1; CMAH; and PPT1;
 qqqq) BAX; BAK; BCKDHA; BCKDHB; ICAM-1; and SIRT-1; or
 rrrr) BAX; BAK; BCKDHA; BCKDHB; and ICAM-1.

is reduced or eliminated.

In certain examples, the present disclosure is directed to the above-described
 15 modified cells, where the one or more endogenous products have no detectable expression.

In certain examples, the present disclosure is directed to the above-described
 modified cells, where the modified cells are transfected to express a recombinant product of
 interest. In certain examples, the present disclosure is directed to the above-described
 modified cells, where the recombinant product of interest comprises a recombinant viral
 20 vector. In certain examples, the present disclosure is directed to the above-described
 modified cells, where the recombinant product of interest comprises a recombinant viral
 particle. In certain examples, the present disclosure is directed to the above-described
 modified cells, where the recombinant product of interest comprises a recombinant protein.
 In certain examples, the present disclosure is directed to the above-described modified cells,
 25 where the recombinant protein is antibody or an antigen-binding fragment thereof. In certain
 examples, the present disclosure is directed to the above-described modified cells, where
 the antibody is a multispecific antibody or an antigen-binding fragment thereof. In certain
 examples, the present disclosure is directed to the above-described modified cells, where
 the antibody consists of a single heavy chain sequence and a single light chain sequence or
 30 antigen-binding fragments thereof. In certain examples, the present disclosure is directed to
 the above-described modified cells, where the antibody is a chimeric antibody, a human
 antibody or a humanized antibody. In certain examples, the present disclosure is directed to
 the above described modified cells, where the antibody is a

monoclonal antibody. In certain examples, the present disclosure is directed to the above-described modified cells, where the exogenous nucleic acid sequence is integrated in the cellular genome of the mammalian cell at one or more targeted locations.

5 In certain examples, the present disclosure is directed to the above-described modified cells, where the modified cell does not express detectable BAX; BAK; ICAM-1; SIRT-1; PERK; MYC; GGTA1; CMAH; LPLA2; PPT1; BCKDHA; BCKDHB; LPL; and/or LIPA. In certain examples, the present disclosure is directed to the above-described modified cells, where the modified cell expresses decreased levels of GAG; BAX; BAK; ICAM-1; PERK; SIRT-1; MYC; GGTA1; CMAH; LPLA2; PPT1; BCKDHA; BCKDHB;
10 LPL; and/or LIPA.

In certain examples, the present disclosure is directed to the above-described modified cells, where the modified cell is a modified mammalian cell. In certain examples, the modified cell is a modified CHO cell. In another example, the modified cell is a modified HEK 293, HEK-293T, BHK, A549 or HeLa cell.

15 In certain examples, the present disclosure is directed to a composition comprising the above-described modified cells. In one aspect, the present disclosure is directed to a composition comprising a modified mammalian cell as disclosed herein.

In certain examples, the present disclosure is directed to a method of producing a recombinant product of interest comprising culturing a modified mammalian cell expressing
20 the recombinant product of interest, wherein the modified cells expressing the recombinant product of interest exhibit reduced or eliminated expression of one or more of the following GAG; BAX; BAK; ICAM-1; PERK; SIRT-1; MYC; GGTA1; CMAH; LPLA2; BCKDHA; BCKDHB; PPT1; LPL; and/or LIPA. In one aspect, the present disclosure is directed to a method of producing a recombinant product of interest comprising: i) culturing a modified
25 mammalian cell as disclosed herein; ii) recovering the recombinant product of interest from the cultivation medium or the modified mammalian cells, wherein the modified cells expressing the recombinant product of interest exhibit reduced or eliminated expression of one or more of the following GAG; BAX; BAK; ICAM-1; SIRT-1; PERK; MYC; GGTA1; CMAH; LPLA2; PPT1; BCKDHA; BCKDHB; LPL; and/or LIPA.

30 In certain examples, the present disclosure is directed to a method of culturing a population of mammalian cells expressing a recombinant product of interest, wherein the modified cells expressing the recombinant product of interest exhibit reduced or eliminated expression of one or more of the following GAG; BAX; BAK; ICAM-1; PERK; SIRT-1; MYC; GGTA1; CMAH; LPLA2; PPT1; BCKDHA; BCKDHB; LPL; and/or LIPA.

35 In certain examples, the present disclosure is directed to a method culturing a population of modified mammalian cells expressing a recombinant product of interest or a method of producing a recombinant product of interest comprising culturing a population of mammalian cells expressing a recombinant product of interest, wherein modified cells expressing the recombinant product of interest exhibit reduced or eliminated expression of:

40 a) BAX; BAK; ICAM-1; GGTA1; CMAH; LPL; LPLA2; and PPT1;

qqqq) BAX; BAK; BCKDHA; BCKDHB; ICAM-1; and SIRT-1; or

rrrr) BAX; BAK; BCKDHA; BCKDHB; and ICAM-1.

In certain examples, the present disclosure is directed to a method culturing a population of modified mammalian cells expressing a recombinant product of interest or a method of producing a recombinant product of interest comprising culturing a population of mammalian cells expressing a recombinant product of interest, wherein modified cells expressing the recombinant product of interest exhibit reduced or eliminated expression of:

- a) GAG; BAX; BAK; ICAM-1; GGTA1; CMAH; LPL; LPLA2; and PPT1;
- b) GAG; BAX; BAK; ICAM-1; SIRT-1; GGTA1; CMAH; LPL; LPLA2; and PPT1;
- 10 c) GAG; BAX; BAK; ICAM-1; SIRT-1; MYC; GGTA1; CMAH; LPL; LPLA2; and PPT1;
- d) GAG; BAX; BAK; ICAM-1; SIRT-1; MYC; GGTA1; CMAH; LPL; LPLA2; PPT1; and LIPA;
- e) GAG; BAX; BAK; ICAM-1; GGTA1; CMAH; LPLA2; PPT1; and LIPA;
- 15 f) GAG; BAX; BAK; ICAM-1; SIRT-1; GGTA1; CMAH; LPLA2; PPT1; and LIPA;
- g) GAG; BAX; BAK; ICAM-1; SIRT-1; MYC; GGTA1; CMAH; LPLA2; PPT1; and LIPA;
- h) GAG; BAX; BAK; ICAM-1; LPL; LPLA2; and PPT1;
- i) GAG; BAX; BAK; ICAM-1; SIRT-1; LPL; LPLA2; and PPT1;
- 20 j) GAG; BAX; BAK; ICAM-1; SIRT-1; MYC; LPL; LPLA2; and PPT1;
- k) GAG; BAX; BAK; ICAM-1; SIRT-1; MYC; LPL; LPLA2; PPT1; and LIPA;
- l) GAG; BAX; BAK; ICAM-1; SIRT-1; and MYC;
- m) GAG; BAX; BAK; ICAM-1; PERK; SIRT-1; and MYC;
- n) GAG; BAX; BAK; ICAM-1; PERK; SIRT-1; MYC; GGTA1; CMAH; LPL; LPLA2; PPT1; and LIPA;
- 25 o) BAX; BAK; LPL; LPLA2; GGTA1; and CMAH;
- p) BAX; BAK; PERK; LPL; LPLA2; GGTA1; and CMAH;
- q) BAX; BAK; MYC; LPL; LPLA2; GGTA1; and CMAH;
- r) BAX; BAK; MYC; PERK; LPL; LPLA2; GGTA1; and CMAH;
- 30 s) GAG; BAX; BAK; LPL; LPLA2; GGTA1; CMAH; and PPT1;
- t) GAG; BAX; BAK; PERK; LPL; LPLA2; GGTA1; CMAH; and PPT1;
- u) GAG; BAX; BAK; MYC; LPL; LPLA2; GGTA1; CMAH; and PPT1;
- v) GAG; BAX; BAK; MYC; PERK; LPL; LPLA2; GGTA1; CMAH; and PPT1;

IIII) BAX; BAK; BCKDHA; BCKDHB; MYC; PERK; LPL; LPLA2; GGTA1; and
 CMAH;
 mmmm) BAX; BAK; BCKDHA; BCKDHB; LPL; LPLA2; GGTA1; CMAH; and
 PPT1;
 5 nnnn) BAX; BAK; BCKDHA; BCKDHB; PERK; LPL; LPLA2; GGTA1;
 CMAH; and PPT1;
 oooo) BAX; BAK; BCKDHA; BCKDHB; MYC; LPL; LPLA2; GGTA1;
 CMAH; and PPT1;
 pppp) BAX; BAK; BCKDHA; BCKDHB; MYC; PERK; LPL; LPLA2;
 10 GGTA1; CMAH; and PPT1;
 qqqq) BAX; BAK; BCKDHA; BCKDHB; ICAM-1; and SIRT-1; or
 rrrr) BAX; BAK; BCKDHA; BCKDHB; and ICAM-1.

In certain examples, the present disclosure is directed to the above-described
 methods for culturing a population of modified mammalian cells expressing a recombinant
 15 product of interest or producing a recombinant product of interest comprising culturing a
 population of mammalian cells expressing a recombinant product of interest, wherein the
 recombinant product of interest is encoded by a nucleic acid sequence. In certain examples,
 the nucleic acid sequence is integrated in the cellular genome of the modified cells at one or
 more targeted locations. In certain examples, the recombinant product of interest expressed
 20 by the modified cells is encoded by a nucleic acid sequence that is randomly integrated in
 the cellular genome of the mammalian cells. In certain examples, the recombinant product
 of interest comprises a recombinant viral vector. In certain examples, the recombinant
 product of interest comprises a recombinant viral particle. In certain examples, the
 recombinant product of interest comprises a recombinant protein. In certain examples, the
 25 recombinant protein is an antibody or an antigen-binding fragment thereof. In certain
 examples, the antibody is a multispecific antibody or an antigen-binding fragment thereof.
 In certain examples, the antibody consists of a single heavy chain sequence and a single
 light chain sequence or antigen-binding fragments thereof. In certain examples, the
 antibody is a chimeric antibody, a human antibody or a humanized antibody. In certain
 30 examples, the antibody is a monoclonal antibody. In certain examples, the methods
 comprise purifying the recombinant product of interest, harvesting the product of interest,
 and/or formulating the product of interest. In certain

examples, the modified cell is a modified CHO cell. In certain examples, the modified cell is a modified HEK 293, HEK 293T, BHK, A549, or HeLa cell

In certain examples, the subject matter of the present disclosure is directed to compositions comprising a modified mammalian cell as described herein.

5 In certain examples, the subject matter of the present disclosure is directed to methods of producing a recombinant product of interest comprising: i) culturing a modified mammalian cell comprising an exogenous nucleic acid encoding a recombinant product of interest as described herein; ii) recovering the recombinant product of interest from a cultivation medium or the modified mammalian cells, wherein the modified cells expressing
10 the recombinant product of interest exhibit reduced or eliminated expression of one or more of the following GAG; BAX; BAK; ICAM-1; SIRT-1; PERK; MYC; GGTA1; CMAH; LPLA2; PPT1; BCKDHA; BCKDHB; LPL; and/or LIPA.

In certain examples, the present disclosure is directed to methods for producing a modified mammalian cell, comprising: applying a nuclease-assisted and/or nucleic acid
15 targeting at least one endogenous gene selected from the group of genes consisting GAG; BAX; BAK; ICAM-1; PERK; SIRT-1; MYC; GGTA1; CMAH; LPLA2; PPT1; BCKDHA; BCKDHB; LPL; and LIPA, in the mammalian cell to reduce or eliminate the expression of said endogenous gene, and selecting the modified mammalian cell wherein the expression of said endogenous gene has been reduced or eliminated as compared to an unmodified
20 mammalian cell. In one aspect, the present disclosure is directed to a method for producing a modified mammalian cell, comprising: (1) applying a nuclease-assisted and/or nucleic acid targeting at least one endogenous gene selected from the group of genes consisting of GAG; BAX; BAK; ICAM-1; PERK; SIRT-1; MYC; GGTA1; CMAH; LPLA2; PPT1; BCKDHA; BCKDHB; LPL; and LIPA, in the mammalian cell to reduce or eliminate the
25 expression of said endogenous gene, and (2) selecting the modified mammalian cell wherein the expression of said endogenous gene has been reduced or eliminated as compared to an unmodified mammalian cell. In certain examples, the nuclease-assisted gene targeting system is selected from the group consisting of CRISPR/Cas9, CRISPR/Cpf1, zinc-finger nuclease, TALEN or meganuclease.

30 In certain examples, the modification to the modified mammalian cells described herein is performed before the introduction of the exogenous nucleic acid encoding the recombinant product of interest, or after the introduction of the exogenous nucleic acid encoding the recombinant product of interest.

In certain examples, the reduction of gene expression in the modified mammalian
35 cells of the present disclosure is mediated by RNA silencing. In certain examples, the RNA silencing is selected from the group consisting of siRNA gene targeting and knockdown, shRNA gene targeting and knockdown, and miRNA gene targeting and knockdown.

In certain examples, the modified cells expressing a recombinant product of interest exhibit reduced or eliminated expression of:

nnnn) BAX; BAK; BCKDHA; BCKDHB; PERK; LPL; LPLA2; GGTA1;
CMAH; and PPT1;

oooo) BAX; BAK; BCKDHA; BCKDHB; MYC; LPL; LPLA2; GGTA1;
CMAH; and PPT1;

5 pppp) BAX; BAK; BCKDHA; BCKDHB; MYC; PERK; LPL; LPLA2;
GGTA1; CMAH; and PPT1;

qqqq) BAX; BAK; BCKDHA; BCKDHB; ICAM-1; and SIRT-1; or

rrrr) BAX; BAK; BCKDHA; BCKDHB; and ICAM-1.

In certain examples, the modified cells expressing the recombinant product of
10 interest exhibit reduced or eliminated expression of:

a) GAG; BAX; BAK; ICAM-1; GGTA1; CMAH; LPL; LPLA2; and PPT1;

b) GAG; BAX; BAK; ICAM-1; SIRT-1; GGTA1; CMAH; LPL; LPLA2; and
PPT1;

c) GAG; BAX; BAK; ICAM-1; SIRT-1; MYC; GGTA1; CMAH; LPL; LPLA2;
15 and PPT1;

d) GAG; BAX; BAK; ICAM-1; SIRT-1; MYC; GGTA1; CMAH; LPL; LPLA2;
PPT1; and LIPA;

e) GAG; BAX; BAK; ICAM-1; GGTA1; CMAH; LPLA2; PPT1; and LIPA;

f) GAG; BAX; BAK; ICAM-1; SIRT-1; GGTA1; CMAH; LPLA2; PPT1; and
20 LIPA;

g) GAG; BAX; BAK; ICAM-1; SIRT-1; MYC; GGTA1; CMAH; LPLA2; PPT1;
and LIPA;

h) GAG; BAX; BAK; ICAM-1; LPL; LPLA2; and PPT1;

i) GAG; BAX; BAK; ICAM-1; SIRT-1; LPL; LPLA2; and PPT1;

25 j) GAG; BAX; BAK; ICAM-1; SIRT-1; MYC; LPL; LPLA2; and PPT1;

k) GAG; BAX; BAK; ICAM-1; SIRT-1; MYC; LPL; LPLA2; PPT1; and LIPA;

l) GAG; BAX; BAK; ICAM-1; SIRT-1; and MYC;

m) GAG; BAX; BAK; ICAM-1; PERK; SIRT-1; and MYC;

n) GAG; BAX; BAK; ICAM-1; PERK; SIRT-1; MYC; GGTA1; CMAH; LPL,
30 LPLA2; PPT1; and LIPA;

o) GAG; BAX; BAK; LPL; LPLA2; GGTA1; and CMAH;

p) GAG; BAX; BAK; PERK; LPL; LPLA2; GGTA1; and CMAH;

q) GAG; BAX; BAK; MYC; LPL; LPLA2; GGTA1; and CMAH;

gggg) BAX; BAK; BCKDHA; BCKDHB; ICAM-1; PERK; SIRT-1; and
MYC;

hhhh) BAX; BAK; BCKDHA; BCKDHB; ICAM-1; PERK; SIRT-1;
MYC; GGTA1; CMAH; LPL, LPLA2; PPT1; and LIPA;

5 iii)BAX; BAK; BCKDHA; BCKDHB; LPL; LPLA2; GGTA1; and CMAH;
jjjj)BAX; BAK; BCKDHA; BCKDHB; PERK; LPL; LPLA2; GGTA1; and CMAH;
kkkk) BAX; BAK; BCKDHA; BCKDHB; MYC; LPL; LPLA2; GGTA1;
and CMAH;

llll)BAX; BAK; BCKDHA; BCKDHB; MYC; PERK; LPL; LPLA2; GGTA1; and
10 CMAH;

mmmm) BAX; BAK; BCKDHA; BCKDHB; LPL;
LPLA2; GGTA1; CMAH; and PPT1;

nnnn) BAX; BAK; BCKDHA; BCKDHB; PERK; LPL; LPLA2; GGTA1;
CMAH; and PPT1;

15 oooo) BAX; BAK; BCKDHA; BCKDHB; MYC; LPL; LPLA2; GGTA1;
CMAH; and PPT1;

pppp) BAX; BAK; BCKDHA; BCKDHB; MYC; PERK; LPL; LPLA2;
GGTA1; CMAH; and PPT1;

qqqq) BAX; BAK; BCKDHA; BCKDHB; ICAM-1; and SIRT-1; or

20 rrrr) BAX; BAK; BCKDHA; BCKDHB; and ICAM-1.

In certain examples of the above-described methods for expressing a recombinant product of interest, the recombinant product of interest is encoded by a nucleic acid sequence. In certain examples, the nucleic acid sequence encoding the recombinant product of interest is integrated in the cellular genome of the modified cells at one or more targeted
25 locations. In certain examples, the nucleic acid sequence encoding the recombinant product of interest is randomly integrated in the cellular genome of the mammalian cells. In certain examples, the nucleic acid sequence encoding the recombinant product of interest is integrated into the cellular genome of a mammalian cell by means of a transposase-mediated gene integration (using, for e.g., Lonza's GS piggyBac transposase system, ATUM's Leap-
30 In transposase system, or Probiogen's DirectedLuck transposase with epigenetic targeting).

In certain examples, the recombinant product of interest comprises a viral vector. In certain examples, the recombinant product of interest comprises a viral particle. In

certain examples, the recombinant product of interest comprises a recombinant protein. In certain examples, the recombinant protein is an antibody or an antigen-binding fragment thereof. In certain examples, antibody is a multispecific antibody or an antigen-binding fragment thereof. In certain examples, the antibody consists of a single heavy chain sequence and a single light chain sequence or antigen-binding fragments thereof. In certain examples, the antibody is a chimeric antibody, a human antibody or a humanized antibody. In certain examples, the antibody is a monoclonal antibody.

In certain examples, the subject matter of the present disclosure comprises purifying the product of interest expressed by a modified mammalian cell as disclosed herein, harvesting the product of interest, and/or formulating the product of interest.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. CRISPR/Cas9 multiplex knockout (KO) method achieves high-efficiency knockouts (confirmed by LC-MS/MS). A schematic displaying the multiplex gene editing approach is illustrated. Individual gRNAs were first screened for each knockout target. The most potent gRNAs were multiplexed with Cas9 protein and transfected into cells sequentially to generate a highly edited pool of cells ($\geq 75\%$ indel frequency). Percent indel was measured at the pool stage of each target to determine the probability of clones with all target genes knocked out. After single cell cloning (SCC), clones were analyzed and screened via PCR and Sanger sequencing to identify those with all targets knocked out. Top clones were selected to initiate production cultures to characterize their growth profiles. At the end of the production culture, the harvested cell culture fluid (HCCF) was submitted for LC-MS/MS for verification of knockouts at the protein level. The top knockout hosts were selected for cryopreservation to create cell banks.

Figure 2. Screening process and indel analysis for detecting knockout efficiencies. Sanger traces generated from workflow illustrated in Figure 1 were analyzed using ICE software (Synthego) to determine editing efficiency.

Figure 3. CRISPR/Cas9 multiplex knockout method achieves high-efficiency knockouts. Comparison of KO efficiency for each gene in the 10x transfected CHO pools. The 10x transfected pools were generated by transfecting 2x KO cells (BAX/BAK double KO cells). The KO targets for the 10x KO cells were BAX, BAK, SIRT-1, MYC, ICAM-1, LPLA2, LPL, PPT1, CMAH, and GGTA1.