(LPL): CHO cell lines with LPL knocked out resulted in cell culture harvests with 41-57% less polysorbate degradation than their wild-type counterpart (Chiu et al., Biotechnol Bioeng, 2017, Volume 114, Issue 5, Pages 1006-1015). By reducing or eliminating the expression of such enzymes, the negative impact of the associated enzymatic activity (e.g., hydrolytic degradation of polysorbate in the drug product by residual hydrolases) may be mitigated. However, that investigation did not determine if the benefits observed for the LPL knock out at the cell culture harvest stage was maintained through downstream processing. It is important to test purified materials generated from knock out cell lines to demonstrate the benefits are achieved after purification and thus translate into benefits in the drug product.

3. SUMMARY

The present disclosure relates to modified mammalian cells, (e.g., Chinese Hamster Ovary (CHO) cells) that have reduced or eliminated activity of certain host cell proteins, e.g., host cell enzymes, including but not limited to, certain lipases, esterases, and/or hydrolases, methods for making such cells, and methods of using such cells in the production of a product of interest, e.g., a recombinant protein.

In one aspect, the present disclosure provides a recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of one or more enzyme relative to the activity of the enzyme in an unmodified cell. In certain examples, the present disclosure provides a recombinant host cell where the cell is modified to reduce or eliminate the activity of one or more enzyme relative to the activity of the enzyme in an unmodified cell. In certain examples, the one or more enzyme is selected from the group consisting of: Lipoprotein lipase (LPL); phospholipase B-domain containing 2 (PLBL2/PLBD2); Lipase A (Lysosomal acid lipase/cholesteryl ester hydrolase, Lipase) (LIPA); Phospholipase A-2-activating protein (PLAA); Phospholipase D3 (PLD3); Phospholipase A2 group XV (LPLA2); Phospholipase C beta 1 (PLCB1); Phospholipase C delta 1 (PLCD1); DDHD domain containing protein 1 (Fragment) (DDHD1); Lysophospholipase-like protein 1 (LYPLAL1); Phospholipase A2 group XIIA (PLA2G12A); Peroxiredoxin 6 (PRDX6); Sphingomyelin phosphodiesterase (SMPD1); Palmitoyl-protein thioesterase 1 (PPT1); Isoamyl acetate hydrolyzing esterase 1 (putative) (IAH1); OTU deubiquitinase, ubiquitin aldehyde binding 1 (OTUB1); Lysophospholipase 2 (Acyl-protein thioesterase 2) (LYPLA2); Acyl-coenzyme A thioesterase 13 (ACOT13); Fatty acid synthase (FASN); Phospholipase A2 group VII (PLA2G7); Ubiquitin specific peptidase 5 (USP5); N-acylsphingosine amidohydrolase 1 (Acid ceramidase) (ASAH1); Lipase maturation factor 1 (LMF1); Apolipoprotein-CII (APOC2); Acylcarnitine hydrolase (HACH);

Carboxylesterase 1F (CES1F) or Liver carboxylesterase B-1-like (CES-B1L); Lysophospholipase 1 (LYPLA1); Carboxylesterase 1 (CES1); Phospholipase A1 member A (PLA1A); and Sialic acid acetylesterase (SIAE).

In certain examples, the activity of: a) PPT1; b) LPLA2; LPL; and LIPA; c) LPLA2; LPL; LIPA; PPT1; PLBL2; PLD3; and SPD1; d) LPLA2; LPL; LIPA; PPT1; PLBL2; PLD3; SPD1; PLAA; IAH1; OTUB1; LYPLA2; and PLA2G12A; e) BAX; BAK; LPLA2; LPL; LIPA; PPT1; PLD3; and SPD1; f) BAX; BAK; LPLA2; LPL; LIPA; PPT1; PLBL2; PLD3; SPD1; CLU; PRDX1; PLAA; and ACOT13; g) LPLA2; LPL; and PPT1; h) LPLA2; LPL; LIPA; and PPT1; i) HACH; CES1F/CES-B1L; and LYPLA1; j) LPLA2; LPL; LIPA; PPT1; HACH; CES1F/CES-B1L; and LYPLA1; k) SMPD1; CES1; PLA1A; and SIAE; l) LPLA2; LPL; LIPA; PPT1; HACH; CES1F/CES-B1L; LYPLA1; SMPD1; CES1; PLA1A; and SIAE; m) LPLA2; LMF1; LIPA; PPT1; HACH; CES1F/CES-B1L; and LYPLA1; n) LPLA2; LMF1; APOC2; LIPA; PPT1; HACH; CES1F/CES-B1L; and LYPLA1; o) LMF1 and APOC2 in a recombinant host cell is reduced or eliminated.

In certain examples, the activity of the one or more enzyme in a recombinant host cell is reduced or eliminated by: (a) knocking down expression of the enzyme; (b) or knocking out expression of the enzyme; or (c) altering the nucleic acid sequence encoding the enzyme.

In certain examples, the present disclosure is directed to a recombinant host cell comprising one or more altered enzyme genes. In certain examples, the one or more altered enzyme genes have no detectable enzymatic activity. In certain examples, the recombinant host cell comprises a nucleic acid sequence encoding a product of interest. In certain examples, the nucleic acid sequence is integrated in the cellular genome of the mammalian cell at a targeted location. In certain examples, the recombinant host cell further comprises a nucleic acid encoding the product of interest that is randomly integrated in the cellular genome of the mammalian cell. In certain examples, the modified cell does not express any detectable LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE.

In one aspect, the present disclosure provides a composition comprising a recombinant host cell as disclosed herein. In certain examples, the present disclosure provides compositions comprising a recombinant host cell described in the present disclosure.

In one aspect, the present disclosure provides an *in vitro* method for reducing enzymatic activity in a cell, comprising: (a) knocking down or knocking out the expression of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE; (b) modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE; (c) selecting cells with reduced activity of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE; or (d) altering the gene encoding one or more of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE.

In certain examples, the present disclosure provides a method for reducing

enzymatic activity in a cell, where the method comprises knocking down or knocking out the expression of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE.

In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, where the method comprises modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE.

In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, wherein the method comprises selecting cells with reduced activity of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE.

In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, wherein the method comprises altering the gene encoding one or more of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE.

In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, wherein the method comprises administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE.

In one aspect, the present disclosure provides an *in vitro* method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding one or more of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; and ASAH1 so that the one or more of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE have reduced or eliminated enzymatic activity.

In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, wherein the method comprises administering to the cell a genetic engineering system, wherein the genetic engineering system alters the

nucleic acid sequence encoding one or more of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE so that the one or more of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; SIAE have reduced or eliminated enzymatic activity.

In one aspect, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of one or more of the following LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE.

In certain examples, the present disclosure provides a method of culturing a population of mammalian cells expressing a product of interest, wherein the mammalian cells have reduced or eliminated activity of one or more of the following LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE.

In certain examples, the present disclosure provides a recombinant host cell comprising one or more altered enzyme genes. In certain examples, the one or more altered enzyme genes are altered by disruption of a coding region. In certain examples, the one or more enzyme genes alteration comprises a biallelic alteration. In certain examples, the one or more enzyme genes alteration comprises a deletion of 1 or more base pairs, 2 or more base pairs, 3 or more base pairs, 4 or more base pairs, 5 or more base pairs, 6 or more base pairs, 7 or more base pairs, 8 or more base pairs, 9 or more base pairs, 10 or more base pairs, 11 or more base pairs, 12 or more base pairs, 13 or more base pairs, 14 or more base pairs, 15 or more base pairs, 16 or more base pairs, 17 or more base pairs, 18 or more base pairs, 19 or more base pairs, or 20 or more base pairs. In certain examples, the one or more enzyme genes are LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1;

PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE.

In certain of the above described examples, the genetic engineering system is selected from the group consisting of a CRISPR/Cas system, a zinc-finger nuclease (ZFN) system, a transcription activator-like effector nuclease (TALEN) system and a combination thereof. In certain of the above described examples, the genetic engineering system is a CRISPR/Cas9 system.

In certain of the above described examples, the CRISPR/Cas9 system comprises: (a) a Cas9 molecule, and (b) one or more guide RNAs (gRNAs) comprising a targeting sequence that is complementary to a target sequence in a gene encoding LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE.

In certain of the above described examples, the genetic engineering system comprises an RNA selected from the group consisting of: a short hairpin RNA (shRNA), a small interference RNA (siRNA), and a microRNA (miRNA), wherein the RNA is complementary to a portion of an mRNA expressed by one or more of the LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE genes. In certain of the above described examples, the genetic engineering system is a zinc-finger nuclease (ZFN) system or a transcription activator-like effector nuclease (TALEN) system.

In certain of the above described examples, the reduction or elimination of activity is of: a) PPT1; b) LPLA2; LPL; and LIPA; c) LPLA2; LPL; LIPA; PPT1; PLBL2; PLD3; and SPD1; d) LPLA2; LPL; LIPA; PPT1; PLBL2; PLD3; SPD1; PLAA; IAH1; OTUB1; LYPLA2; and PLA2G12A; e) BAX; BAK; LPLA2; LPL; LIPA; PPT1; PLD3; and SPD1; f) BAX; BAK; LPLA2; LPL; LIPA; PPT1; PLBL2; PLD3; SPD1; CLU; PRDX1; PLAA; and ACOT13 or g) LPLA2; LPL; and PPT1; h) LPLA2; LPL; LIPA; and PPT1; i) HACH; CES1F/CES-B1L; and LYPLA1; j) LPLA2; LPL; LIPA; PPT1; HACH; CES1F/CES-B1L; and LYPLA1; k) SMPD1; CES1; PLA1A; and SIAE; l) LPLA2; LPL; LIPA; PPT1; HACH; CES1F/CES-B1L; LYPLA1; SMPD1; CES1; PLA1A; and SIAE; m) LPLA2; LMF1; LIPA; PPT1; HACH; CES1F/CES-B1L; and LYPLA1; n) LPLA2;

LMF1; APOC2; LIPA; PPT1; HACH; CES1F/CES-B1L; and LYPLA1; o) LMF1 and APOC2 in the mammalian cells.

In certain of the above described examples, the methods provided in the present disclosure further comprise purifying the product of interest, harvesting the product of interest, and/or formulating the product of interest.

In certain of the above described examples, the degradation of a polyoxyethylene sorbitan monolaurate is reduced. In certain of the above described examples, the degradation of polysorbate 20 (PS20 or Tween 20) is reduced. In certain of the above described examples, the degradation of polysorbate 80 (PS80 or Tween 80) is reduced.

In certain of the above described examples, the cell is a mammalian cell. In certain of the above described examples, the mammalian cell is a CHO cell.

In certain of the above described examples, the cell expresses a product of interest. In certain of the above described examples, the product of interest expressed by the mammalian cells is encoded by a nucleic acid sequence. In certain of the above described examples, the nucleic acid sequence is integrated in the cellular genome of the mammalian cells at a targeted location. In certain of the above described examples, the product of interest expressed by the cells is further encoded by a nucleic acid sequence that is randomly integrated in the cellular genome of the mammalian cells.

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

In certain examples, the modified cell of the present disclosure does not express any detectable PPT1. In certain examples, the modified cell of the present disclosure does not express any detectable LPLA2, LPL, and LIPA. In certain examples, the modified cell of the present disclosure does not express any detectable

LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, and SMPD1. In certain examples, the modified cell of the present disclosure does not express any detectable LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, PLAA, IAH1, OTUB1, LYPLA2, and PLA2G12A. In certain examples, the modified cell of the present disclosure does not express any detectable BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLD3, and SMPD1. In certain examples, the modified cell of the present disclosure does not express any detectable BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, CLU, PRDX1, PLAA, and ACOT13. In certain examples, the modified cell of the present disclosure does not express any detectable LPLA2, LPL, and PPT1. In certain examples, the modified cell of the present disclosure does not express any detectable LPLA2, LPL, LIPA and PPT1. In certain examples, the modified cell of the present disclosure does not express any detectable HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the modified cell of the present disclosure does not express any detectable LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the modified cell of the present disclosure does not express any detectable SMPD1, CES1, PLA1A, and SIAE. In certain examples, the modified cell of the present disclosure does not express any detectable LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, LYPLA1, SMPD1, CES1, PLA1A, and SIAE. In certain examples, the modified cell of the present disclosure does not express any detectable LPLA2, LMF1, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the modified cell of the present disclosure does not express any detectable LPLA2, LMF1, APOC2, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the modified cell of the present disclosure does not express any detectable LMF1 and APOC2.

In certain examples, the present disclosure provides a recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of PPT1 enzymes relative to the activity of the enzymes in an unmodified cell. In certain examples, the present disclosure provides a recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of LPLA2, LPL, and LIPA enzymes relative to the activity of the enzymes in an unmodified cell. In certain examples, the present disclosure provides a recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, and SMPD1 enzymes relative to the activity of the enzymes in an unmodified cell. In certain examples, the present disclosure provides a recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, PLAA, IAH1, OTUB1,

LYPLA2, and PLA2G12A enzymes relative to the activity of the enzymes in an unmodified cell. In certain examples, the present disclosure provides a recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLD3, and SMPD1 enzymes relative to the activity of the enzymes in an unmodified cell. In certain examples, the present disclosure provides a recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, CLU, PRDX1, PLAA, and ACOT13 enzymes relative to the activity of the enzymes in an unmodified cell. In certain examples, the present disclosure provides a recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of LPLA2, LPL, and PPT1 enzymes relative to the activity of the enzymes in an unmodified cell. In certain examples, the present disclosure provides a recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of LPLA2, LPL, LIPA and PPT1 enzymes relative to the activity of the enzymes in an unmodified cell. In certain examples, the present disclosure provides recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of HACH, CES1F/CES-B1L, and LYPLA1 enzymes relative to the activity of the enzymes in an unmodified cell. In certain examples, the present disclosure provides a recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1 enzymes relative to the activity of the enzymes in an unmodified cell. In certain examples, the present disclosure provides a recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of SMPD1, CES1, PLA1A, and SIAE enzymes relative to the activity of the enzymes in an unmodified cell. In certain examples, the present disclosure provides a recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, LYPLA1, SMPD1, CES1, PLA1A, and SIAE enzymes relative to the activity of the enzymes in an unmodified cell. In certain examples, the present disclosure provides a recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of LPLA2, LMF1, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1 enzymes relative to the activity of the enzymes in an unmodified cell. In certain examples, the present disclosure provides a recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of LPLA2, LMF1, APOC2, LIPA, PPT1, HACH, CES1F/CES-B1L and LYPLA1 enzymes relative to the activity of the enzymes in an unmodified cell. In certain examples, the present disclosure provides a recombinant host cell, wherein the cell is modified to reduce

or eliminate the activity of LMF1 and APOC2 relative to their activity in an unmodified cell.

In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising knocking down or knocking out the expression of PPT1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising knocking down or knocking out the expression of LPLA2, LPL, and LIPA. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising knocking down or knocking out the expression of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, and SMPD1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising knocking down or knocking out the expression of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, PLAA, IAH1, OTUB1, LYPLA2, and PLA2G12A. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising knocking down or knocking out the expression of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLD3, and SMPD1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising knocking down or knocking out the expression of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, CLU, PRDX1, PLAA, and ACOT13. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising knocking down or knocking out the expression of LPLA2, LPL, and PPT1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising knocking down or knocking out the expression of LPLA2, LPL, LIPA and PPT1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising knocking down or knocking out the expression of HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising knocking down or knocking out the expression of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising knocking down or knocking out the expression of SMPD1, CES1, PLA1A, and SIAE. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising knocking down or knocking out the expression of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, LYPLA1, SMPD1, CES1, PLA1A, and SIAE. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell,

comprising knocking down or knocking out the expression of LPLA2, LMF1, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising knocking down or knocking out the expression of LPLA2, LMF1, APOC2, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising knocking down or knocking out the expression of LMF1 and APOC2.

In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of PPT1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of LPLA2, LPL, and LIPA. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, and SMPD1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, PLAA, IAH1, OTUB1, LYPLA2, and PLA2G12A. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLD3, and SMPD1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, CLU, PRDX1, PLAA, and ACOT13. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of LPLA2, LPL, and PPT1. In certain examples, the present

disclosure provides a method for reducing enzymatic activity in a cell, comprising modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of LPLA2; LPL; LIPA and PPT1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of SMPD1, CES1, PLA1A, and SIAE. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, LYPLA1, SMPD1, CES1, PLA1A, and SIAE. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of LPLA2, LMF1, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of LPLA2, LMF1, APOC2, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of LMF1 and APOC2.

In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising selecting cells with reduced activity of PPT1. In certain examples, the present disclosure provides a method for reducing enzymatic

activity in a cell, comprising selecting cells with reduced activity of LPLA2, LPL, and LIPA. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising selecting cells with reduced activity of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, and SMPD1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising selecting cells with reduced activity of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, PLAA, IAH1, OTUB1, LYPLA2, and PLA2G12A. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising selecting cells with reduced activity of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLD3, and SMPD1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising selecting cells with reduced activity of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, CLU, PRDX1, PLAA, and ACOT13. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising selecting cells with reduced activity of LPLA2, LPL, and PPT1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising selecting cells with reduced activity of LPLA2, LPL, LIPA and PPT1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising selecting cells with reduced activity of HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising selecting cells with reduced activity of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising selecting cells with reduced activity of SMPD1, CES1, PLA1A, and SIAE. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising selecting cells with reduced activity of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, LYPLA1, SMPD1, CES1, PLA1A, and SIAE. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising selecting cells with reduced activity of LPLA2, LMF1, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising selecting cells with reduced activity of LPLA2, LMF1, APOC2, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising selecting cells with reduced activity of LMF1 and APOC2.

In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising altering the gene encoding PPT1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising altering the gene encoding one or more of LPLA2, LPL, and LIPA. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising altering the gene encoding one or more of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, and SMPD1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising altering the gene encoding one or more of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, PLAA, IAH1, OTUB1, LYPLA2, and PLA2G12A. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising altering the gene encoding one or more of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLD3, and SMPD1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising altering the gene encoding one or more of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, CLU, PRDX1, PLAA, and ACOT13. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising altering the gene encoding one or more of LPLA2, LPL, and PPT1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising altering the gene encoding one or more of LPLA2, LPL, LIPA and PPT1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising altering the gene encoding one or more of HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising altering the gene encoding one or more of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising altering the gene encoding one or more of SMPD1, CES1, PLA1A, and SIAE. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising altering the gene encoding one or more of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, LYPLA1, SMPD1, CES1, PLA1A, and SIAE. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising altering the gene encoding one or more of LPLA2, LMF1, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising altering the gene encoding one or more

of LPLA2, LMF1, APOC2, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing enzymatic activity in a cell, comprising altering the gene encoding one or more of LMF1 and APOC2.

In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of PPT1. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of LPLA2, LPL, and LIPA. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, and SMPD1. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, PLAA, IAH1, OTUB1, LYPLA2, and PLA2G12A. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLD3, and SMPD1. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, CLU, PRDX1, PLAA, and ACOT13. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of LPLA2, LPL, and PPT1. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of LPLA2, LPL, LIPA and PPT1. In certain examples, the present disclosure provides a method for reducing or eliminating

enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of SMPD1, CES1, PLA1A, and SIAE. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, LYPLA1, SMPD1, CES1, PLA1A, and SIAE. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of LPLA2, LMF1, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of LPLA2, LMF1, APOC2, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system knocks down or knocks out the expression of LMF1 and APOC2.

In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding PPT1 so that the PPT1 has reduced or eliminated enzymatic activity. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding LPLA2, LPL, and LIPA so that the LPLA2, LPL, and LIPA have

reduced or eliminated enzymatic activity. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, and SMPD1 so that the LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, and SMPD1 have reduced or eliminated enzymatic activity. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, PLAA, IAH1, OTUB1, LYPLA2, and PLA2G12A so that the LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, PLAA, IAH1, OTUB1, LYPLA2, and PLA2G12A have reduced or eliminated enzymatic activity. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLD3, and SMPD1 so that the BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLD3, and SMPD1 have reduced or eliminated enzymatic activity. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, CLU, PRDX1, PLAA, and ACOT13 so that the BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, CLU, PRDX1, PLAA, and ACOT13 have reduced or eliminated enzymatic activity. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding LPLA2, LPL, and PPT1 so that the LPLA2, LPL, and PPT1 have reduced or eliminated enzymatic activity. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding LPLA2, LPL, LIPA and PPT1 so that the LPLA2, LPL, LIPA and PPT1 have reduced or eliminated enzymatic activity. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic

engineering system alters the nucleic acid sequence encoding HACH, CES1F/CES-B1L, and LYPLA1 so that the HACH, CES1F/CES-B1L, and LYPLA1 have reduced or eliminated enzymatic activity. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1 so that the LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1 have reduced or eliminated enzymatic activity. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding SMPD1, CES1, PLA1A, and SIAE and PPT1 so that the SMPD1, CES1, PLA1A, and SIAE have reduced or eliminated enzymatic activity. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, LYPLA1, SMPD1, CES1, PLA1A, and SIAE so that the LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, LYPLA1, SMPD1, CES1, PLA1A, and SIAE have reduced or eliminated enzymatic activity. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding LPLA2, LMF1, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1 so that the LPLA2, LMF1, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1 have reduced or eliminated enzymatic activity. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding LPLA2, LMF1, APOC2, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1 so that the LPLA2, LMF1, APOC2, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1 have reduced or eliminated enzymatic activity. In certain examples, the present disclosure provides a method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding LMF1 and APOC2 so that the LMF1 and APOC2 have reduced or eliminated activity.

In certain examples, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of PPT1. In certain examples, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of LPLA2, LPL, and LIPA. In certain examples, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, and SMPD1. In certain examples, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, PLAA, IAH1, OTUB1, LYPLA2, and PLA2G12A. In certain examples, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLD3, and SMPD1. In certain examples, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, CLU, PRDX1, PLAA, and ACOT13. In certain examples, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of LPLA2, LPL, and PPT1. In certain examples, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of LPLA2, LPL, LIPA and PPT1. In certain examples, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of HACH, CES1F/CES-B1L, and

LYPLA1. In certain examples, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of SMPD1, CES1, PLA1A, and SIAE. In certain examples, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, LYPLA1, SMPD1, CES1, PLA1A, and SIAE. In certain examples, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of LPLA2, LMF1, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of LPLA2, LMF1, APOC2, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of LMF1 and APOC2.

In certain examples, the present disclosure provides a method of culturing a population of mammalian cells expressing a product of interest, wherein the mammalian cells have reduced or eliminated activity of PPT1. In certain examples, the present disclosure provides a method of culturing a population of mammalian cells expressing a product of interest, wherein the mammalian cells have reduced or eliminated activity of LPLA2, LPL, and LIPA. In certain examples, the present disclosure provides a method of culturing a population of mammalian cells expressing a product of interest, wherein the mammalian cells have reduced or eliminated activity of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, and SMPD1. In certain examples, the present disclosure provides a method of culturing a population of mammalian cells expressing a product of

interest, wherein the mammalian cells have reduced or eliminated activity of LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, PLAA, IAH1, OTUB1, LYPLA2, and PLA2G12A. In certain examples, the present disclosure provides a method of culturing a population of mammalian cells expressing a product of interest, wherein the mammalian cells have reduced or eliminated activity of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLD3, and SMPD1. In certain examples, the present disclosure provides a method of culturing a population of mammalian cells expressing a product of interest, wherein the mammalian cells have reduced or eliminated activity of BAX, BAK, LPLA2, LPL, LIPA, PPT1, PLBL2, PLD3, SMPD1, CLU, PRDX1, PLAA, and ACOT13. In certain examples, the present disclosure provides a method of culturing a population of mammalian cells expressing a product of interest, wherein the mammalian cells have reduced or eliminated activity of LPLA2, LPL, and PPT1. In certain examples, the present disclosure provides a method of culturing a population of mammalian cells expressing a product of interest, wherein the mammalian cells have reduced or eliminated activity of LPLA2, LPL, LIPA and PPT1. In certain examples, the present disclosure provides a method of culturing a population of mammalian cells expressing a product of interest, wherein the mammalian cells have reduced or eliminated activity of HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method of culturing a population of mammalian cells expressing a product of interest, wherein the mammalian cells have reduced or eliminated activity of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method of culturing a population of mammalian cells expressing a product of interest, wherein the mammalian cells have reduced or eliminated activity of SMPD1, CES1, PLA1A, and SIAE. In certain examples, the present disclosure provides a method of culturing a population of mammalian cells expressing a product of interest, wherein the mammalian cells have reduced or eliminated activity of LPLA2, LPL, LIPA, PPT1, HACH, CES1F/CES-B1L, LYPLA1, SMPD1, CES1, PLA1A, and SIAE. In certain examples, the present disclosure provides a method of culturing a population of mammalian cells expressing a product of interest, wherein the mammalian cells have reduced or eliminated activity of LPLA2, LMF1, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present disclosure provides a method of culturing a population of mammalian cells expressing a product of interest, wherein the mammalian cells have reduced or eliminated activity of LPLA2, LMF1, APOC2, LIPA, PPT1, HACH, CES1F/CES-B1L, and LYPLA1. In certain examples, the present

WHAT IS CLAIMED IS:

1. A recombinant host cell, wherein the cell is modified to reduce or eliminate the activity of one or more enzyme relative to the activity of the enzyme in an unmodified cell.

2. The recombinant host cell of claim 1, wherein the one or more enzyme is selected from the group consisting of: Lipoprotein lipase (LPL); phospholipase B-domain containing 2 (PLBL2/PLBD2); Lipase A (Lysosomal acid lipase/cholesteryl ester hydrolase, Lipase) (LIPA); Phospholipase A-2-activating protein (PLAA); Phospholipase D3 (PLD3); Phospholipase A2 group XV (LPLA2); Phospholipase C beta 1 (PLCB1); Phospholipase C delta 1 (PLCD1); DDHD domain containing protein 1 (Fragment) (DDHD1); Lysophospholipase-like protein 1 (LYPLAL1); Phospholipase A2 group XIIA (PLA2G12A); Peroxiredoxin 6 (PRDX6); Sphingomyelin phosphodiesterase (SMPD1); Palmitoyl-protein thioesterase 1 (PPT1); Isoamyl acetate hydrolyzing esterase 1 (putative) (IAH1); OTU deubiquitinase, ubiquitin aldehyde binding 1 (OTUB1); Lysophospholipase 2 (Acyl-protein thioesterase 2) (LYPLA2); Acyl-coenzyme A thioesterase 13 (ACOT13); Fatty acid synthase (FASN); Phospholipase A2 group VII (PLA2G7); Ubiquitin specific peptidase 5 (USP5); N-acylsphingosine amidohydrolase 1 (Acid ceramidase) (ASAH1); Lipase Maturation Factor 1 (LMF1); Apolipoprotein C-II (APOC2); Acylcarnitine hydrolase (HACH); Carboxylesterase 1F (CES1F) or Liver carboxylesterase B-1-like (CES-B1L); Lysophospholipase 1 (LYPLA1); Carboxylesterase 1 (CES1); Phospholipase A1 member A (PLA1A); and Sialic acid acetylesterase (SIAE).

3. The recombinant host cell of claim 2, wherein the activity of:

a) PPT1;

b) LPLA2; LPL; and LIPA;

c) LPLA2; LPL; LIPA; PPT1; PLBL2; PLD3; and SMPD1;

d) LPLA2; LPL; LIPA; PPT1; PLBL2; PLD3; SMPD1; PLAA; IAH1; OTUB1; LYPLA2; and PLA2G12A;

e) BAX; BAK; LPLA2; LPL; LIPA; PPT1; PLD3; and SMPD1;

f) BAX; BAK; LPLA2; LPL; LIPA; PPT1; PLBL2; PLD3; SMPD1; CLU; PRDX1; PLAA; and ACOT13;

g) LPLA2; LPL; and PPT1

h) LPLA2; LPL; LIPA and PPT1

i) HACH; CES1F/CES-B1L; and LYPLA1

j) LPLA2; LPL; LIPA; PPT1; HACH; CES1F/CES-B1L; and LYPLA1

k) SMPD1; CES1; PLA1A; and SIAE

l) LPLA2; LPL, LIPA; PPT1; HACH; CES1F/CES-B1L; LYPLA1; SMPD1; CES1; PLA1A; and SIAE

m) LPLA2; LMF1; LIPA; PPT1; HACH; CES1F/CES-B1L; and LYPLA1

n) LPLA2; LMF1; APOC2; LIPA; PPT1; HACH; CES1F/CES-B1L; and LYPLA1 or

o) LMF1 and APOC2

is reduced or eliminated.

4. The recombinant host cell of claim 1, wherein the activity of the one or more enzyme is reduced or eliminated by:

(a) knocking down expression of the enzyme;

(b) or knocking out expression of the enzyme; or

(c) altering the nucleic acid sequence encoding the enzyme.

5. The recombinant host cell of claim 1, wherein the cell comprises one or more altered enzyme genes.

6. The recombinant host cell of claim 5, wherein the one or more altered enzyme genes have no detectable enzymatic activity.

7. The recombinant host cell of claim 1, wherein the cell is a mammalian cell.

8. The recombinant host cell of claim 7, wherein the cell is a CHO cell.

9. The recombinant host cell of claim 1, comprising a nucleic acid sequence encoding a product of interest.

10. The recombinant host cell of claim 9, wherein the product of interest comprises a protein, a viral particle or a viral vector.

11. The recombinant host cell of claim 9, wherein the product of interest comprises a recombinant protein.

12. The recombinant host cell of claim 9, wherein the product of interest comprises an antibody or an antigen-binding fragment thereof.

13. The recombinant host cell of claim 9, wherein the nucleic acid sequence is integrated in the cellular genome of the mammalian cell at a targeted location.

14. The recombinant host cell of claim 13, further comprising a nucleic acid encoding the product of interest that is randomly integrated in the cellular genome of the mammalian cell.

15. The recombinant host cell of claim 1, wherein the modified cell does not express any detectable LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2;

ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE.

16. A composition comprising a recombinant host cell of claim 1.

17. An *in vitro* method for reducing enzymatic activity in a cell, comprising: (a) knocking down or knocking out the expression of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE; (b) modulating a cell culture process and/or media composition, wherein modulating a cell culture process and/or media composition results in reduced activity of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE; (c) selecting cells with reduced activity of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE; or (d) altering the gene encoding one or more of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE.

18. An *in vitro* method for reducing or eliminating enzymatic activity in a cell, comprising administering to the cell a genetic engineering system, wherein the genetic engineering system alters the nucleic acid sequence encoding one or more of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; and ASAH1 so that the one or more of LPL; PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE have reduced or eliminated enzymatic activity.

19. A method of producing a product of interest comprising culturing mammalian cells expressing the product of interest, wherein the mammalian cells express the product of interest and have reduced or eliminated activity of one or more of the following LPL;

PLBL2/PLBD2; LIPA; PLAA; PLD3; LPLA2; PLCB1; PLCD1; DDHD1; LYPLAL1; PLA2G12A; PRDX6; SMPD1; PPT1; IAH1; OTUB1; LYPLA2; ACOT13; FASN; PLA2G7; USP5; ASAH1; LMF1; APOC2; HACH; CES1F/CES-B1L; LYPLA1; CES1; PLA1A; and/or SIAE.

20. The method of claim 19, wherein the degradation of polyoxyethylene sorbitan monolaurate is reduced.

21. The method of claim 19, wherein the degradation of polysorbate 20 (PS20 or Tween 20) is reduced.

22. The method of claim 19, wherein the degradation of polysorbate 80 (PS80 or Tween 80) is reduced.