

expressing the recombinant product of interest exhibit improved attributes relevant to cell viability and the titer associated with production of products of interest. Such improved cells can be achieved by modifying the genome of the cells (i.e., cell line engineering).

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3. SUMMARY

In one aspect, the present disclosure is directed to a modified cell, wherein the cell is modified to reduce or eliminate the expression of two or more endogenous proteins relative to the expression of the endogenous proteins in an unmodified cell, wherein: (a) one or more of the endogenous proteins having reduced or eliminated expression promotes
10 apoptosis of the modified cell during cell culture; and (b) one or more of the endogenous proteins having reduced or eliminated expression regulates the unfolded protein response (UPR).

In one aspect, the present disclosure is directed to a modified cell, wherein the cell is modified to reduce or eliminate the expression of two or more endogenous proteins
15 relative to the expression of the endogenous proteins in an unmodified cell, wherein one or more endogenous proteins is selected from the endogenous protein group consisting of: BCL2 Associated X, Apoptosis Regulator (BAX); and BCL2 Antagonist/Killer 1 (BAK); and one of the endogenous proteins is Protein Kinase R-like ER Kinase (PERK).

In certain examples, the present disclosure is directed to a modified cell,
20 wherein the expression of BAX, BAK, and PERK is reduced or eliminated.

In certain examples, the present disclosure is directed to the above described modified cell, where the modified cell is engineered to express a recombinant product of interest. In certain examples, the present disclosure is directed to the above described modified cell, where the modified cell is generated from a recombinant cell that expresses
25 a recombinant product of interest. In certain examples, the present disclosure is directed to the above described modified cell, where the one or more endogenous proteins have no detectable expression. In certain examples, the present disclosure is directed to the above described modified cell, where the recombinant product of interest comprises a viral vector. In certain examples, the present disclosure is directed to the above described modified cell,
30 where the recombinant product of interest comprises a viral particle. In certain examples, the present disclosure is directed to the above described modified cell, where the recombinant product of interest comprises a recombinant protein. In certain examples, the present disclosure is directed to the above described modified cell, where

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the recombinant protein is antibody or an antibody-fusion protein or an antigen-binding fragment thereof. In certain examples, the present disclosure is directed to the above described modified cell, 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 cell, where the antibody consists of a single heavy chain sequence and a single light chain sequence or antigen-binding fragments thereof. In certain examples, the present disclosure is directed to the above described modified cell, 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 cell, where the antibody is a monoclonal antibody. In certain examples, the present disclosure is directed to the above described modified cell, where the recombinant product of interest is encoded by an exogenous nucleic acid sequence integrated in the cellular genome at one or more targeted locations.

In certain examples, the present disclosure is directed to the above described modified cell, where the modified cell does not express detectable BAX, BAK, and PERK. In certain examples, the present disclosure is directed to the above described modified cell, where the modified cell expresses decreased levels of BAX, BAK, and PERK.

In certain examples, the present disclosure is directed to the above described modified cell, where the modified cell is a modified animal cell. In certain examples, the modified animal cell is a modified Sf9, CHO, HEK 293, HEK-293T, BHK, A549, or HeLa cell.

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 cell as disclosed herein.

In certain examples, the present disclosure is directed to a method of producing a recombinant product of interest comprising: (a) culturing the above described modified cell; and (b) recovering the recombinant product of interest from a cultivation medium or the modified cells, wherein the modified cells expressing the recombinant product of interest exhibit reduced or eliminated expression of BAX, BAK, and PERK. In one aspect, the present disclosure is directed to a method of producing a recombinant product of interest comprising: (a) culturing a modified cell as disclosed herein; and (b) recovering the recombinant product of interest from a cultivation medium or the modified cells, wherein the modified cells expressing the recombinant product of interest exhibit reduced or eliminated expression of BAX, BAK, and PERK.

In one aspect, the present disclosure is directed to a method for producing a modified cell, comprising: (a) applying a nuclease-assisted and/or nucleic acid targeting BAX, BAK, and PERK, in the cell to reduce or eliminate the expression of said endogenous genes, and (b) selecting the modified cell wherein the expression of said endogenous genes have been reduced or eliminated as compared to an unmodified cell.

In certain examples of the above-described methods for producing a

modified cell, the modification 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 nuclease-assisted gene targeting system is selected from the group consisting of
5 CRISPR/Cas9, CRISPR/Cpf1, zinc-finger nuclease, TALEN or meganuclease. In certain examples, the reduction of gene expression is mediated by RNA silencing. In certain examples, the RNA silencing is selected from the group consisting of siRNA gene targeting and knock-down, shRNA gene targeting and knock-down, and miRNA gene targeting and knock-down.

10 In certain examples, 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 by the modified cells is encoded by a nucleic acid sequence that is randomly integrated in the cellular genome of the modified cells.

15 In certain examples, the recombinant product of interest comprises a viral vector. In certain examples, 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 antibody-fusion protein or an antigen-binding fragment thereof. In certain examples, the antibody is a multispecific
20 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.

25 In certain examples of the above-described methods comprise purifying the product of interest, harvesting the product of interest, and/or formulating the product of interest.

In certain examples of the above-described methods the modified cell is a modified animal cell. In certain examples, the modified animal cell is a modified Sf9, CHO,
30 HEK 293, HEK 293T, BHK, A549, or HeLa cell.

In certain examples, the present disclosure is directed to modified cell that has a higher specific productivity than a corresponding isolated animal cell that comprises the polynucleotide and functional copies of each of the wild type Bax, Bak, and PERK

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genes.

In certain examples, the present disclosure is directed to modified cell that is more resistant to apoptosis than a corresponding isolated animal cell that comprises functional copies of each of the Bax, Bak, and PERK genes.

5 In certain examples, the present disclosure is directed to modified cell that is employed in fed-batch, perfusion, process intensified, semi-continuous perfusion, or continuous perfusion cell culture process.

4. BRIEF DESCRIPTION OF THE DRAWINGS

10 **Figure 1.** PDGFRa is down-regulated by UPR activation. Figure 1A and Figure 1B depict that PDGFRa protein levels and mRNA levels, respectively, were downregulated when mAb1-expressing CHO cells are grown at pH 7.07. Figure 1C depicts western blot analysis of two mAb1-expressing host cell lines, CHO DG44 and CHO-K1, treated with chemical UPR inducers: tunicamycin and DTT. Figure 1D depicts qPCR
15 analysis of PDGFRa mRNA levels in both host cell lines of Figure 1C treated with tunicamycin and DTT. Figure 1E depicts western blot analysis of mAb1-expressing CHO-K1 cells treated with tunicamycin to activate the UPR in the presence of UPR pathway-specific inhibitors. RT-PCR panel for XBP-1 shows IRE1alpha RNase activation. Figure 1F depicts qPCR analysis of PDGFRa mRNA levels in CHO-K1 cells treated with tunicamycin
20 in the presence of UPR pathway-specific inhibitors. Figure 1G depicts western blot analysis of WT and PERK KO empty host CHO-K1 (Clone 9) cell lines treated with tunicamycin and PERK inhibitor.

Figure 2. Figure 2A depicts western blot analysis of mAb1-expressing CHO-K1 cells treated with thapsigargin to activate the UPR in the presence of different UPR
25 pathway-specific inhibitors. RT-PCR panel of XBP-1 shows IRE1alpha RNase activation. Figure 2B depicts western blot analysis of empty host CHO-K1 cells treated with Tunicamycin to activate the UPR in the presence of different UPR pathway-specific inhibitors. Figure 2C depicts qPCR analysis of PDGFRa mRNA levels in CHO-K1 cells treated with thapsigargin in the presence of different UPR pathway-specific inhibitors.
30 Figure 2D depicts western blot analysis of Cas9-sgRNAs against the PERK gene with a sgRNA against luciferase as control. Figure 2E depicts western blot analysis of empty host CHO-K1 single cell clones after using Cas9 to knockout PERK. Clone 9 was used in Figure 1G.

Figure 3. PDGFRa signaling is important for cell growth, e.g., CHO cell