under conditions suitable for production of the polypeptide. In some examples, the polynucleotide that encodes the recombinant polypeptide is an extrachromosomal polynucleotide. In some examples, the polynucleotide that encodes the recombinant polypeptide is integrated into a chromosome of the HEK293 cell line. In some examples, the recombinant polypeptide is an antibody or antigen-binding fragment thereof, an antigen, an enzyme, or a vaccine. In some examples, the recombinant polypeptide is an antibody or antigen-binding fragment thereof (*e.g.*, a diagnostic or therapeutic antibody or antigen-binding fragment thereof). In some examples, the cell line produces the recombinant polypeptide at a titer of about 650 mg/L in 7 days. In some examples, the methods further comprise isolating the recombinant polypeptide from the cell line.

[0009a] In one aspect, the present disclosure refers to a method of producing a recombinant polypeptide, comprising:
(i) ~~culturing~~ transfecting a HEK293 cell line that comprises ~~(a)~~ a loss-of-function mutation in each of the human *Bax* and *Bak* genes ~~and (b)~~with a polynucleotide encoding the recombinant polypeptide by contacting the HEK293 cell line with the polynucleotide and polyethylenimine (PEI) to produce a transfected cell line, and (ii) culturing the transfected cell line under conditions suitable for production of the polypeptide, wherein the loss of function mutation is a deletion in each of the *Bax* and *Bak* genes, wherein a PEI:DNA ratio of 7.5 and a DNA concentration of 1 µg/mL was used for transfection.

[0010] In another example, provided herein are methods of producing a viral vector, comprising culturing a HEK293 cell line that comprises (a) a loss-of-function mutation in each of the human *Bax* and *Bak* genes, (b) a viral genome, and (c) one or more polynucleotides encoding a viral capsid under conditions suitable for production of the viral vector. In some examples, the methods further comprise isolating the viral vector from the cell line.

[0011] In some examples of any of the above examples, the cell line is cultured in a cell culture medium. In some examples, the cell line is cultured at a pH of between about 6.7 and about 7.3, between about 6.9 and about 7.1, between about 6.95 and about 7.05, or about 7.0. In some examples, the cell line is cultured with a dissolved oxygen (DO) setpoint of about 30%. In some examples, the cell line is cultured at an agitation rate that imparts a power input per volume (P/V) of about 13W/m^3. In some examples, the cell line is cultured in a volume of at least about 10L. In some examples, the cell line is cultured in a volume of at least about 25L. In some examples, the cell line is cultured in a 35L bioreactor. In some examples, the methods comprise culturing the cell line in a 35L bioreactor for 60 days. In some examples, the cell line maintains at least 85% cell viability after culturing the cell line for 60 days in a 35L bioreactor. In some examples, the cell line is cultured in the 35L bioreactor at a working volume of between about 20L and about 35L. In some examples, the cell line is cultured under fed-batch culture conditions. In some examples, the cell line is cultured under perfusion culture conditions.

[0012] In another example, provided herein are cell cultures comprising a cell culture medium and a plurality of HEK293 cells, wherein each cell of the plurality comprises a loss-of-function

mutation in each of the human *Bax* and *Bak* genes. In some examples, the cells are at a cell density sufficient for a 35L bioreactor culture. In some examples, the cells are maintained at a cell density sufficient for a 35L bioreactor culture for 60 days. In some examples, the cell line maintains at least 85% cell viability after culturing the cell line for 60 days in a 35L bioreactor culture. In some examples, the plurality of cells maintains greater than 75% cell viability after exposure to a shear stress of 2.67×10^7 W/m^3 energy dissipation rate (EDR). In some examples, the plurality of cells maintains greater than 75% cell viability after exposure to 1 μM staurosporine for 70 hours. In some examples, each cell of the plurality comprises a deletion in each of the *Bax* and *Bak* genes. In some examples, the cell line further comprises a polynucleotide that encodes a recombinant polypeptide. In some examples, the polynucleotide that encodes the recombinant polypeptide is an extrachromosomal polynucleotide. In some examples, the polynucleotide that encodes the recombinant polypeptide is integrated into a chromosome of the human cell line. In some examples, the recombinant polypeptide is an antibody or antigen-binding fragment thereof, an antigen, an enzyme, or a vaccine. In some examples, the recombinant polypeptide is an antibody or antigen-binding fragment thereof (*e.g.*, a diagnostic or therapeutic antibody or antigen-binding fragment thereof). In some examples, the cell culture produces the recombinant polypeptide at a titer of about 650 mg/L in 7 days. In some examples, the cell line further comprises a recombinant polynucleotide.

[0012a] In one aspect, the present disclosure refers to a cell culture comprising from 10 L to 35 L of a cell culture medium and a plurality of cells of a HEK293 cell~~s~~ lines, wherein each cell of the plurality comprises a loss-of-function mutation in each of the human *Bax* and *Bak* genes, and wherein the loss of function mutation is a deletion in each of the *Bax* and *Bak* genes.

[0013] In another example, provided herein is a HEK293 cell line (*e.g.*, an isolated HEK293 cell line) that comprises a loss-of-function mutation in each of the human *Bax* and *Bak* genes. In some examples, the cell line comprises a deletion in each of the *Bax* and *Bak* genes. In some examples, the cell line further comprises a viral genome and one or more polynucleotides encoding a viral capsid. In some examples, the cell line further comprises a polynucleotide encoding a recombinant polypeptide. In some examples, the polynucleotide that encodes the recombinant polypeptide is an extrachromosomal polynucleotide. In some examples, the polynucleotide that encodes the recombinant polypeptide is integrated into a chromosome of the human cell line. In some examples, the recombinant polypeptide is an antibody or antigen-binding fragment thereof, an antigen, an enzyme, or a vaccine. In some examples, the recombinant polypeptide is an antibody or antigen-binding fragment thereof (*e.g.*, a diagnostic or therapeutic antibody or antigen-binding fragment thereof). In some examples, the cell line

produces a higher titer of the recombinant polypeptide than a corresponding isolated human cell line that comprises the polynucleotide and functional copies of each of the human *Bax* and *Bak* genes. In some examples, the cell line is more resistant to shear stress than a corresponding isolated human cell line that comprises functional copies of each of the human *Bax* and *Bak* genes. In some examples, the cell line is more resistant to apoptosis than a corresponding isolated human cell line that comprises functional copies of each of the human *Bax* and *Bak* genes. In some examples, the cell line is more resistant to staurosporine than a corresponding isolated human cell line that comprises functional copies of each of the human *Bax* and *Bak* genes.

~~**[0013a]** In one aspect, the present disclosure refers to an isolated HEK293 cell line, wherein the cell line comprises a loss-of-function mutation in each of the human *Bax* and *Bak* genes, and wherein the loss-of-function mutation is a deletion in each of the *Bax* and *Bak* genes.~~

[0014] In another example, provided herein are cell cultures comprising the cell line according to any one of the above examples and a cell culture medium.

[0015] It is to be understood that one, some, or all of the properties of the various examples described herein may be combined to form other examples of the present disclosure. These and other examples of the disclosure will become apparent to one of skill in the art. These and other examples of the disclosure are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] **FIGS. 1A-1D** compare the HEK293 DKO cell line (with *Bax* and *Bak* genes knocked out as described in Example 1) to the parental HEK293 cell line. **FIG. 1A** shows cell viability after exposure of the cell lines to 1 µM staurosporine to induce apoptosis. **FIGS. 1B-1D** show the effects of using a flow constriction device (FCD) to assess sensitivity of the DKO and parental cell lines to shear stress on total lysis after FCD (**FIG. 1B**), viable cell density (VCD) before and after FCD (**FIG. 1C**), and viability before and after FCD (**FIG. 1D**).

[0017] **FIGS. 2A-2C** show the optimization of N:P ratio and DNA concentration for HEK293 DKO transient transfections. Transfections were tested across N:P ratios of 5 to 12.5 and DNA concentrations of 0.75 to 1.5 µg/mL (**FIG. 2A**). **FIGS. 2B & 2C** show the effects of transfecting HEK293 and HEK293 DKO cells at the 30 mL tubespin scale with an N:P ratio of 7.5 and a DNA concentration of 1 µg/mL on viable cell density (VCD) (**FIG. 2B**) and viability over the 7 day production cultures and day 7 titers (**FIG. 2C**).

CLAIMS

What is claimed is:

1. A method of producing a recombinant polypeptide, comprising:

i) transfecting~~culturing~~ a HEK293 cell line that comprises ~~(a)~~ a loss-of-function mutation in each of the human *Bax* and *Bak* genes with ~~and (b)~~ a polynucleotide encoding the recombinant polypeptide by contacting the HEK293 cell line with the polynucleotide and polyethylenimine (PEI) to produce a transfected cell line, and

ii) culturing the transfected cell line under conditions suitable for production of the polypeptide,

wherein the loss of function mutation is a deletion in each of the *Bax* and *Bak* genes, wherein a PEI:DNA ratio of 7.5 and a DNA concentration of 1 µg/mL was used for transfection.

2. The method of claim 1, wherein the polynucleotide that encodes the recombinant polypeptide is an extrachromosomal polynucleotide.

~~3. The method of claim 1, wherein the polynucleotide that encodes the recombinant polypeptide is integrated into a chromosome of the HEK293 cell line.~~

~~4.~~3. The method of any one of claims 1-2, wherein the recombinant polypeptide is an antibody or antigen-binding fragment thereof, an antigen, an enzyme, or a vaccine.

~~5.~~4. The method of claim ~~4~~3, wherein the transfected cell line produces the recombinant polypeptide at a titer of about 650 mg/L in 7 days.

~~6.~~5. The method of claim 3 or claim ~~5~~4, wherein the transfected cell line is cultured:

(a) at a pH of between ~~about~~ 6.7 and ~~about~~ 7.3; and/or

(b) with a dissolved oxygen (DO) setpoint of about 30%.; and/or

(c) at an agitation rate that imparts a power input per volume (P/V) of about 13W/m^3.

~~7.~~6. The method of claim ~~4~~5, wherein the cell line maintains at least 85% cell viability after culturing the cell line for 60 days in a 35L bioreactor culture.

~~8.~~7. The method of claim 4~~5~~, wherein the cell line is cultured in the 35L bioreactor culture at a working volume of between ~~about~~ 20L and ~~about~~ 35L.

~~9.~~8. The method of claim 4~~5~~, wherein the cell line is cultured under fed-batch culture conditions.

~~10.~~9. The method of claim 4~~5~~, wherein the cell line is cultured under perfusion culture conditions.

~~11.~~10. A cell culture comprising from 10 L to 35 L of a cell culture medium and a plurality of cells of a HEK293 cell line~~s~~, wherein each cell of the plurality comprises a loss-of-function mutation in each of the human *Bax* and *Bak* genes, and wherein the loss of function mutation is a deletion in each of the *Bax* and *Bak* genes.

~~12.~~11. The cell culture of claim ~~11~~10, wherein the cell line maintains at least 85% cell viability after culturing the cell line for 60 days in a 35L bioreactor culture.

~~13.~~12. The cell culture of claim ~~11~~10, wherein the ~~plurality of cells~~cell line maintains greater than 75% cell viability after exposure to a shear stress of 2.67×10^7 W/m^3 energy dissipation rate (EDR).

~~14.~~13. The cell culture of claim ~~11~~10, wherein the ~~plurality of cells~~cell line maintains greater than 75% cell viability after exposure to 1 µM staurosporine for 70 hours.

~~15. An isolated HEK293 cell line, wherein the cell line comprises a loss-of-function mutation in each of the human *Bax* and *Bak* genes, and wherein the loss-of-function mutation is a deletion in each of the *Bax* and *Bak* genes.~~

~~16.~~14. The cell culture~~line~~ of any one of claims 10-13~~15~~, wherein the cell line further comprises a polynucleotide encoding a recombinant polypeptide, and wherein the polynucleotide is DNA.

~~17.~~15. The cell culture~~line~~ of claim 14~~16~~, wherein the polynucleotide that encodes the recombinant polypeptide is an extrachromosomal polynucleotide.

~~18.~~16. The cell culture~~line~~ of claim 14~~16~~, wherein the polynucleotide that encodes the recombinant polypeptide is integrated into a chromosome of the ~~human~~ cell line.

~~19.~~17. The cell culture~~line~~ of any one of claims 14-16, wherein the recombinant polypeptide is an antibody or antigen-binding fragment thereof.

~~20.~~18. The cell culture~~line~~ of claim 17~~16~~, wherein the cell line produces a higher titer of the recombinant polypeptide than a corresponding isolated human cell line that comprises the polynucleotide and functional copies of each of the human *Bax* and *Bak* genes.

~~21.~~19. The cell culture~~line~~ of claim 17 or claim 18~~16~~, wherein the cell line is more resistant to shear stress than a corresponding isolated human cell line that comprises functional copies of each of the human *Bax* and *Bak* genes.

~~22.~~20. The cell culture~~line~~ of any one of claims 17-19~~16~~, wherein the cell line is more resistant to apoptosis than a corresponding isolated human cell line that comprises functional copies of each of the human *Bax* and *Bak* genes.

~~23.~~21. The cell culture~~line~~ of any one of claims 17-20~~16~~, wherein the cell line is more resistant to staurosporine than a corresponding isolated human cell line that comprises functional copies of each of the human *Bax* and *Bak* genes.

22. The cell culture of any one of claims 10-21, wherein the cell culture is contained within a stirred tank bioreactor.

23. The cell culture of any one of claims 10-21, wherein the cell culture is contained within a wavebag bioreactor.