production systems result in low titer yields, manufacturing sufficient levels of rAAV for use in human trials and commercial applications is challenging. Researchers have explored numerous ways to generate adequately high titers of rAAV particles, but there is still a great need for addressing this issue. In particular, there is a need for efficient cell lines that are able to produce high quality rAAV with high titer yields. Production of high titer rAAV by the engineered cell lines described herein expedites the application of this vector system for gene therapy use *in vivo*.

SUMMARY

[0005] The present disclosure addresses the need for obtaining improved rAAV titers for gene therapy applications by providing rAAV packaging and/or producer cell lines comprising cells in which one or more genes and/or proteins have been modified. Also described herein are methods of identifying one or more genes and/or proteins that are relevant to the production of rAAV, and methods of generating engineered rAAV packaging and/or producer cell lines.

[0005a] In one aspect of the present disclosure, there is provided a recombinant adeno-associated virus (rAAV) packaging and/or producer cell line comprising cells which have been engineered to reduce the expression and/or activity of a gene product expressed from ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1 as compared to corresponding unmodified parental cells.

[0005b] In another aspect of the present disclosure, there is provided a recombinant adeno-associated virus (rAAV) packaging and/or producer cell line, wherein said cell line comprises cells which exhibit reduced expression and/or activity of a polypeptide or polyribonucleotide expressed from at least one of ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and NALCN-AS1 as compared to a corresponding parental cell line.

[0005c] In a further aspect of the present disclosure, there is provided a lysate or a cell culture supernatant of the cell line disclosed herein.

[0005d] In another aspect of the present disclosure, there is provided a method of identifying one or more genes relevant to the production of rAAV, the method comprising:

i. adding one or more supplements that increase the rAAV titer in a cell line;

ii. measuring the global gene expression across the transcriptome in supplemented and non-supplemented cell lines;

iii. obtaining a list of genes that are differentially expressed between supplemented and non-supplemented cell lines; and

iv. identifying one or more genes that are relevant to the production of rAAV.

[0005e] In a further aspect of the present disclosure, there is provided a method of producing a rAAV packaging and/or producer cell line to promote increased production of rAAV, the method comprising modulating the expression of one or more genes identified using the method disclosed herein.

[0006] Described herein are compositions and methods of generating rAAV packaging and/or producer cell lines comprising cells that can produce a higher titer of rAAV compared to control parental cells. More specifically, provided herein are rAAV packaging and/or producer cell lines comprising cells in which expression of one or more genes and/or proteins is modulated resulting in a higher rAAV titer compared to control parental cells. In one example, the present disclosure provides rAAV packaging and/or producer cell lines comprising cells in which expression of one or more genes and/or proteins is reduced compared to control parental cells. For example, expression of ATP5EP2 (ATP Synthase F1 Subunit Epsilon Pseudogene 2), LINC00319 (Long Intergenic Non-Protein Coding RNA 319), CYP3A7 (Cytochrome P450 Family 3 Subfamily A Member 7), ABCA10 (ATP Binding Cassette Subfamily A Member 10), NOG (Noggin), RGMA (Repulsive Guidance Molecule BMP Co-Receptor A), SPANXN3 (SPANX Family Member N3), PGA5 (Pepsinogen A5), MYRIP (Myosin VIIA And Rab Interacting Protein), KCNN2 (Potassium Calcium-Activated Channel Subfamily N Member 2), and/or NALCN-AS1 (NALCN Antisense RNA 1) is reduced compared to control parental cells.

[0007] In some examples, the present disclosure provides rAAV packaging and/or producer cell lines comprising cells in which expression of KCNN2, LINC00319, RGMA, and SPANXN3 is reduced compared to control parental cells.

[0008] In certain examples, the present disclosure provides a rAAV packaging and/or producer cell line comprising cells which have been engineered to reduce the expression and/or activity of a gene product expressed from ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1 as compared to corresponding unmodified parental cells. In certain examples, the present disclosure provides a rAAV packaging and/or producer cell line that exhibits reduced expression and/or activity of a polypeptide or a polyribonucleotide expressed from at least one of ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and NALCN-AS1 as compared to a corresponding parental cell line.

[0009] In one example, the present disclosure provides a rAAV packaging and/or producer cell line in which expression of one or more genes is reduced using a nuclease, a double stranded RNA (dsRNA), a small interfering RNA (siRNA), a small hairpin RNA (shRNA), a microRNA (miRNA), or an antisense RNA oligonucleotide (ASO).

[0010] In certain examples, the expression of one or more genes is reduced with an siRNA comprising a nucleotide sequence selected from any one of sequences SEQ ID NOs: 1-11. For example, in some examples, expression of ATP5EP2 is reduced, and the siRNA comprises the nucleotide sequence of SEQ ID NO: 1 in the sense strand and the nucleotide sequence of SEQ ID NO: 32 in the anti-sense strand. In some examples, expression of LINC00319 is reduced, and the siRNA comprises the nucleotide sequence of SEQ ID NO: 2 in the sense strand and the nucleotide sequence of SEQ ID NO: 33 in the anti-sense strand. In some examples, expression of CYP3A7 is reduced, and the siRNA comprises the nucleotide sequence of SEQ ID NO: 3 in the sense strand and the nucleotide sequence of SEQ ID NO: 34 in the anti-sense strand. In some examples, expression of NOG is reduced, and the siRNA comprises the nucleotide sequence of SEQ ID NO: 4 in the sense strand and the nucleotide sequence of SEQ ID NO: 35 in the anti-sense strand. In some examples, expression of SPANXN3 is reduced, and the siRNA comprises the nucleotide sequence of SEQ ID NO: 5 in the sense strand and the nucleotide sequence of SEQ ID NO: 36 in the anti-sense strand. In some

examples, expression of MYRIP is reduced, and the siRNA comprises the nucleotide sequence of SEQ ID NO: 6 in the sense strand and the nucleotide sequence of SEQ ID NO: 37 in the anti-sense strand. In some examples, expression of KCNN2 is reduced, and the siRNA comprises the nucleotide sequence of SEQ ID NO: 7 in the sense strand and the nucleotide sequence of SEQ ID NO: 38 in the anti-sense strand. In some examples, expression of NALCN-AS1 is reduced, and the siRNA comprises the nucleotide sequence of SEQ ID NO: 8 in the sense strand and the nucleotide sequence of SEQ ID NO: 39 in the anti-sense strand. In some examples, expression of RGMA is reduced, and the siRNA comprises the nucleotide sequence of SEQ ID NO: 9 in the sense strand and the nucleotide sequence of SEQ ID NO: 40 in the anti-sense strand. In some examples, expression of PGA5 is reduced, and the siRNA comprises the sequence of SEQ ID NO: 10 in the sense strand and the sequence of SEQ ID NO: 41 in the anti-sense strand. In some examples, expression of ABCA10 is reduced, and the siRNA comprises the sequence of SEQ ID NO: 11 in the sense strand and the sequence of SEQ ID NO: 42 in the anti-sense strand.

[0011] In certain examples, the nuclease used to reduce expression of one or more genes is selected from the group consisting of a Zinc Finger nuclease (ZFN), a meganuclease, a transcription activator-like effector nuclease (TALEN), or a clustered regularly interspaced short palindromic repeats (CRISPR) associated protein.

[0012] In certain examples, the expression of one or more genes is reduced using CRISPR genome editing. In some examples, a guide RNA pair is used to target a gene to reduce and/or eliminate expression of that gene. In certain examples, the expression of one or more genes is reduced using a guide RNA pair, wherein each guide RNA: (a) comprises a sequence selected from the nucleotide sequences of SEQ ID NOs: 12-15 and/or (b) targets a target DNA sequence selected from any one of the nucleotide sequences of SEQ ID NO: 16-31. For example, in some examples, the gRNA pair is used to target KCNN2 and comprises a first gRNA molecule comprising the sequence of SEQ ID NO: 12 and a second gRNA molecule comprising the sequence of SEQ ID NO: 13. In some examples, the gRNA pair is used to target KCNN2 and comprises a first gRNA molecule comprising the sequence of SEQ ID NO: 14 and a second gRNA molecule comprising the sequence of SEQ ID NO: 15. In some examples, each gRNA

molecule is a 2' O-methyl analog comprising 3' phosphorothioate internucleotide linkages in the terminal three nucleotides on either or both its 5' and 3' ends.

[0013] In certain examples, one guide RNA pair is used to reduce expression of one gene. In certain other examples, multiple guide RNA pairs are used to reduce expression of one or more genes. In certain examples, the gene expression of one or more genes and/or the activity of one or more genes and/or proteins is reduced and/or eliminated in a rAAV packaging and/or producer cell line compared to a control parental cell line. In certain examples, the gene expression and/or activity is eliminated in the rAAV packaging and/or producer cells compared to control parental cells.

[0014] In some examples described herein, the rAAV packaging and/or producer cell line is a eukaryotic cell line. In certain examples, the rAAV packaging and/or producer cell line is a human cell line. In certain examples, the rAAV packaging and/or producer cell line is an insect cell line. In certain examples, the rAAV packaging and/or producer cell line is a HeLa cell line. In certain other examples, the rAAV packaging and/or producer cell line is a human embryonic kidney (HEK) 293 cell line.

[0015] In some examples described herein, the rAAV packaging and/or producer cell line of the present disclosure produces a higher rAAV titer than a control parental cell line. In certain examples, the titer of rAAV produced from cells of the rAAV producer cell line of the present disclosure is increased about 1.5 to about 7 fold compared to the titer of rAAV produced from a cell line comprising the control parental cells. Also described herein are lysate of the engineered cell lines. In certain examples, higher titer rAAV is harvested from a lysate. Also described herein are cell culture supernatants from engineered cell lines. In certain examples, higher titer rAAV is harvested from a cell culture supernatant.

[0016] Also described herein is a method of generating a producer cell line where the method includes delivering a rAAV vector to cells of a packaging cell line in which the expression of ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1 is reduced compared to control parental cells. In certain examples, the present disclosure provides a method of generating a producer cell line, where the method includes delivering a rAAV vector to cells of a

packaging cell line in which the expression of KCNN2, LINC00319, RGMA, and SPANXN3 is reduced compared to control parental cell.

[0017] Also described herein is a method of producing rAAV by infecting the cells of a producer cell line, generated by a packaging cell line, with a helper virus, wherein the expression of ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1 is reduced in the packaging cell line compared to control parental cells. In certain examples, the expression of KCNN2, LINC00319, RGMA, and SPANXN3 is reduced in the packaging cell line compared to control parental cells.

[0018] In one example, the present disclosure provides a method of producing rAAV, by infecting the cells of a producer cell line with a helper virus, wherein the expression of ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1 is reduced in the producer cell line compared to control parental cells. In certain examples, the present disclosure provides a method of producing rAAV, by infecting the cells of a producer cell line with a helper virus, wherein the expression of KCNN2, LINC00319, RGMA, and SPANXN3 is reduced in the producer cell line compared to control parental cells.

[0019] Also described herein is a method of harvesting rAAV from a producer cell line in which the expression of ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1 is reduced compared to control parental cell line. Also described is a method of harvesting rAAV from a producer cell line in which the expression of KCNN2, LINC00319, RGMA, and SPANXN3 is reduced compared to control parental cell line. In certain examples, the production of rAAV from a producer cell line of the present disclosure is enhanced compared to a control parental cell line.

[0020] Also described herein is a method of identifying one or more genes relevant to the production of rAAV, where the method includes i.) adding one or more supplements that increase the rAAV titer in a cell line; ii.) measuring the global gene expression across the transcriptome in supplemented and non-supplemented cell lines; iii.) obtaining a list of genes that are differentially expressed between supplemented and non-supplemented cell lines; and iv.) identifying one or more genes that are relevant to the production of

rAAV. In some examples, the one or more identified gene(s) is responsible for reducing the production of rAAV.

[0021] Also described herein is a method of producing a rAAV packaging and/or producer cell line to promote increased production of rAAV. In some examples, rAAV production is increased by modulating the expression of one or more genes and/or proteins identified from a list of genes that are differentially expressed between supplemented and non-supplemented rAAV producer cell lines. In certain examples, rAAV titer is increased by modulating the expression of one or more genes and/or proteins identified from a list of genes that are differentially expressed between supplemented and non-supplemented rAAV producer cell line. In some examples, the modulation of one or more genes and/or proteins increases rAAV titer at least 1.5 fold compared to rAAV titer of a cell line without the modulation. In certain examples, modulating the expression is reduction of expression of one or more genes. In certain examples, modulating the expression comprises reduction of expression of one or more proteins. In certain examples, modulating the expression is elimination of expression of one or more genes. In certain examples, modulating the expression comprises elimination of expression of one or more proteins.

[0022] In some examples, the rAAV packaging and/or producer cell line is a eukaryotic cell line. In certain examples, the cell line is a human cell line. In certain examples, the cell line is an insect cell line. In certain examples, the cell line is a HeLa cell line. In certain examples, the cell line is a human embryonic kidney (HEK) 293 cell line.

[0023] Also described herein is a recombinant adeno-associated virus (rAAV) packaging and/or producer cell line comprising cells which have been engineered to reduce the expression and/or activity of a gene product expressed from ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1 as compared to corresponding unmodified parental cells.

[0024] In some examples, the expression and/or activity of a gene product expressed from ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1 is reduced indefinitely or permanently.

[0025] In some examples, the cell line has been engineered to comprise a gene disruption or a partial or complete gene deletion in at least one of ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1.

[0026] In some examples, the cell line has been engineered to comprise a gene disruption in at least one of ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1.

[0027] In some examples, the cell line has been engineered to comprise a gene disruption in at least two genes selected from ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and NALCN-AS1.

[0028] In some examples, the cell line has been engineered to comprise a partial or complete gene deletion in at least one of ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1.

[0029] In some examples, the cell line has been engineered to comprise a partial or complete gene deletion in at least two genes selected from ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and NALCN-AS1.

[0030] Also provided is a packaging and/or producer cell line, wherein said cell line exhibits reduced expression and/or activity of a polypeptide or polyribonucleotide expressed from at least one of ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and NALCN-AS1 as compared to a corresponding parental cell line.

[0031] Other features and advantages of the disclosure will be apparent from the following detailed description and claims.

[0032] Unless noted to the contrary, all publications, references, patents and/or patent applications reference herein are hereby incorporated by reference in their entirety for all purposes.

WHAT IS CLAIMED IS:

1. A recombinant adeno-associated virus (rAAV) packaging and/or producer cell line comprising cells which have been engineered to reduce the expression and/or activity of a gene product expressed from ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1 as compared to corresponding unmodified parental cells.

2. The rAAV packaging and/or producer cell line of claim 1, wherein the expression and/or activity of a gene product expressed from ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1 is reduced indefinitely or permanently.

3. The rAAV packaging and/or producer cell line of claim 2, comprising cells which have been engineered to comprise a gene disruption or a partial or complete gene deletion in at least one of ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1.

4. The rAAV packaging and/or producer cell line of claim 3, comprising cells which have been engineered to comprise a gene disruption in at least one of ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1.

5. The rAAV packaging and/or producer cell line of claim 3, comprising cells which have been engineered to comprise a gene disruption in at least two genes selected from ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and NALCN-AS1.

6. The rAAV packaging and/or producer cell line of claim 3, comprising cells which have been engineered to comprise a partial or complete gene deletion in at least one of ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and/or NALCN-AS1.

7. The rAAV packaging and/or producer cell line of claim 3, comprising cells which have been engineered to comprise a partial or complete gene deletion in at least two genes

selected from ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and NALCN-AS1.

8. The rAAV packaging and/or producer cell line according to one of claims 1-7, wherein the expression and/or activity is reduced using clustered regularly interspaced short palindromic repeat (CRISPR) genome editing.

9. The rAAV packaging and/or producer cell line according to claim 8, wherein the expression is reduced using a guide RNA pair, wherein each guide RNA:

a. comprises a sequence selected from the nucleotide sequences of SEQ ID NOs: 12-15, and/or

b. targets a target DNA sequence selected from any one of the nucleotide sequences of SEQ ID NO: 16-31.

10. The rAAV packaging and/or producer cell line according to claim 9, wherein the gRNA pair is used to target KCNN2 and comprises a first gRNA molecule comprising the sequence of SEQ ID NO: 12 and a second gRNA molecule comprising the sequence of SEQ ID NO: 13; or wherein the gRNA pair is used to target KCNN2 and comprises a first gRNA molecule comprising the sequence of SEQ ID NO: 14 and a second gRNA molecule comprising the sequence of SEQ ID NO: 15.

11. The rAAV packaging and/or producer cell line of claim 10, wherein each gRNA molecule is a 2’ O-methyl analog comprising 3’ phosphorothioate internucleotide linkages in the terminal three nucleotides on either or both its 5’ and 3’ ends.

12. The rAAV packaging and/or producer cell line according to claim 3, wherein the cell line is a human cell line, optionally a HeLa cell line or a human embryonic kidney (HEK) 293 cell line.

13. A recombinant adeno-associated virus (rAAV) packaging and/or producer cell line, wherein said cell line comprises cells which exhibit reduced expression and/or activity of a polypeptide or polyribonucleotide expressed from at least one of ATP5EP2, LINC00319, CYP3A7, ABCA10, NOG, RGMA, SPANXN3, PGA5, MYRIP, KCNN2, and NALCN-AS1 as compared to a corresponding parental cell line.

14. The rAAV packaging and/or producer cell line according to claim 13, wherein the expression and/or activity is reduced using a nuclease, a double stranded RNA (dsRNA), a small interfering RNA (siRNA), a small hairpin RNA (shRNA), a microRNA (miRNA), or an antisense RNA oligonucleotide (ASO).

15. The rAAV packaging and/or producer cell line according to claim 13, wherein:

a. expression and/or activity of ATP5EP2 is reduced using an siRNA, and the siRNA comprises the nucleotide sequence of SEQ ID NO: 1 in the sense strand and the nucleotide sequence of SEQ ID NO: 32 in the anti-sense strand;

b. expression and/or activity of LINC00319 is reduced using an siRNA, and the siRNA comprises the nucleotide sequence of SEQ ID NO: 2 in the sense strand and the nucleotide sequence of SEQ ID NO: 33 in the anti-sense strand;

c. expression and/or activity of CYP3A7 is reduced using an siRNA, and the siRNA comprises the nucleotide sequence of SEQ ID NO: 3 in the sense strand and the nucleotide sequence of SEQ ID NO: 34 in the anti-sense strand;

d. expression and/or activity of NOG is reduced using an siRNA, and the siRNA comprises the nucleotide sequence of SEQ ID NO: 4 in the sense strand and the nucleotide sequence of SEQ ID NO: 35 in the anti-sense strand;

e. expression and/or activity of SPANXN3 is reduced using an siRNA, and the siRNA comprises the nucleotide sequence of SEQ ID NO: 5 in the sense strand and the nucleotide sequence of SEQ ID NO: 36 in the anti-sense strand;

f. expression and/or activity of MYRIP is reduced using an siRNA, and the siRNA comprises the nucleotide sequence of SEQ ID NO:6 in the sense strand and the nucleotide sequence of SEQ ID NO: 37 in the anti-sense strand;

g. expression and/or activity of KCNN2 is reduced using an siRNA, and the siRNA comprises the nucleotide sequence of SEQ ID NO:7 in the sense strand and the nucleotide sequence of SEQ ID NO: 38 in the anti-sense strand;

h. expression and/or activity of NALCN-AS 1 is reduced using an siRNA, and the siRNA comprises the nucleotide sequence of SEQ ID NO: 8 in the sense strand and the nucleotide sequence of SEQ ID NO: 39 in the anti-sense strand;

i. expression and/or activity of RGMA is reduced using an siRNA, and the siRNA comprises the nucleotide sequence of SEQ ID NO:9 in the sense strand and the nucleotide sequence of SEQ ID NO: 40 in the anti-sense strand;

j. expression and/or activity of PGA5 is reduced using an siRNA, and the siRNA comprises the sequence of SEQ ID NO: 10 in the sense strand and the sequence of SEQ ID NO: 41 in the anti-sense strand; and/or

k. expression and/or activity of ABCA10 is reduced using an siRNA, and the siRNA comprises the sequence of SEQ ID NO: 11 in the sense strand and the sequence of SEQ ID NO: 42 in the anti-sense strand.

16. A lysate or a cell culture supernatant of the cell line according to any one of claims 3 or 13-15.

17. A method of identifying one or more genes relevant to the production of rAAV, the method comprising:

i. adding one or more supplements that increase the rAAV titer in a cell line;

ii. measuring the global gene expression across the transcriptome in supplemented and non-supplemented cell lines;

iii. obtaining a list of genes that are differentially expressed between supplemented and non-supplemented cell lines; and

iv. identifying one or more genes that are relevant to the production of rAAV.

18. The method of claim 17, wherein the one or more supplements added to the cell line comprise dexamethasone, hydrocortisone, prednisolone, methylprednisolone, betamethasone, cortisone, prednisone, budesonide, or triamcinolone.

19. A method of producing a rAAV packaging and/or producer cell line to promote increased production of rAAV, the method comprising modulating the expression of one or more genes identified using the method of claim 17.

20. The method of any one of claims 17-19, wherein the cell line is a human cell line, optionally a HeLa cell line or a human embryonic kidney (HEK) 293 cell line.