CLAIMS

1. ~~A method of expressing at least one α galactosidase A (α-Gal A) protein in a cell, the method comprising administering an~~An expression construct comprising a mutated Woodchuck Hepatitis Virus (WHV) Posttranscriptional Regulatory Element (WPRE) sequence, ~~optionally a mut6 mutated WRPE sequence,~~ and ~~a GLA~~ an α galactosidase A (α-Gal A) transgene encoding at least one α-Gal A protein ~~to the cell such that the α-Gal A protein is expressed in the cell~~.

2. The ~~method~~ expression construct of claim 1, wherein the expression construct comprises a wild-type α-Gal A ~~GLA~~ sequence or a codon optimized α-Gal A ~~GLA~~ sequence.

3. The ~~method~~ expression construct of claim 1 or 2, wherein the expression construct further comprises one or more of the following: an enhancer, a promoter, an intron, ~~a sequence encoding~~ a signal peptide and/or a polyadenylation (polyA) signal, sequence wherein the mutated WPRE sequence, ~~optionally the mut6 mutated WRPE sequence,~~ and the α-Gal A ~~GLA~~ transgene encoding at least one α-Gal A protein are~~is~~ located between the signal peptide and the ~~sequence encoding the polyadenylation~~ polyA signal sequence.

4. The expression construct of any one of claims 1-3, wherein the mutated WPRE sequence is a mut6 mutated WRPE sequence.

5. The expression construct of any one of claims 1-4, wherein the expression construct comprises an apolipoprotein E (APOE) enhancer operably linked to an alpha 1-antitrypsin (hAAT) promoter, a human hemoglobin beta (HBB)-IGG intron, an α-Gal A signal peptide, the mutated WPRE sequence, and a bovine growth hormone poly A signal sequence.

64. The ~~method~~ expression construct of claim 3, wherein the expression construct comprises one or more of the following: the enhancer comprising the nucleotide sequence as set forth in SEQ ID NO: 2, the promoter comprising the nucleotide sequence as set forth in SEQ ID NO: 3, the intron comprising the nucleotide sequence as set forth in SEQ ID NO: 4, the α-GalA transgene comprising nucleic acids 1052-2341 of SEQ ID NO: 9, the mutated WPRE sequence comprising the nucleotide sequence as set forth in SEQ ID NO: 6, and/or the poly A signal sequence comprising the nucleotide sequence as set forth in SEQ ID NO: 7~~; optionally wherein the expression construct comprises the nucleotide sequence of as set forth in SEQ ID NOo: 9~~.

7. The expression construct of any one of claims 1-6, wherein the expression construct comprises the nucleotide sequence as set forth in SEQ ID NO: 9.

~~5. The method of any one of claims 1-4, wherein the cell is in a subject with Fabry's disease.~~

~~6. The method of any one of claims 1-5, wherein the cell is in a male subject.~~

~~7. The method of any one of claims 1-6, wherein the expression construct is administered in a pharmaceutically acceptable carrier.~~

~~8. The method of claim 7, wherein the pharmaceutically acceptable carrier comprises phosphate buffered saline containing $CaCl_2$, $Mg\ Cl_2$, NaCl, sucrose and Kolliphor (Poloxamer) P 188.~~

~~9. The method of any one of claims 1-8, wherein the expression construct sequence comprises the sequence as shown in Table 1 and wherein the expression construct is delivered to the cell by an AAV viral vector.~~

~~10~~8. The ~~method of claim 9~~expression construct of any one of claims 1-7, wherein the expression construct is an adeno-associated virus (AAV) expression construct ~~the AAV viral vector serotype is AAV2/6~~.

9. The expression construct of claim 8, wherein the AAV expression construct serotype is AAV2/6.

10. An expression construct comprising an enhancer comprising the nucleotide sequence as set forth in SEQ ID NO: 2, a promoter comprising the nucleotide sequence as set forth in SEQ ID NO: 3, an intron comprising the nucleotide sequence as set forth in SEQ ID NO: 4, an α-GalA transgene comprising nucleic acids 1052-2341 of SEQ ID NO: 9, a mutated WPRE sequence comprising the nucleotide sequence as set forth in SEQ ID NO: 6, and a poly A signal sequence comprising the nucleotide sequence as set forth in SEQ ID NO: 7.

~~11. The method of any one of claims 5-10, wherein the expression construct is administered to the subject at a dose of between about 5.0E+12 and 1.0E+14 vector genomes per kilogram (vg/kg).~~

~~12. The method of any one of claims 5-11, wherein the expression construct is administered to the liver of the subject.~~

11. A composition, comprising the expression construct of any one of claims 1-10.

12. The composition of claim 11, further comprising a pharmaceutically acceptable carrier.

13. The composition of claim 12, wherein the pharmaceutically acceptable carrier comprises $CaCl_2$, $MgCl_2$, NaCl, Sucrose, and Kolliphor (Poloxamer) P 188.

14. An *in vitro* method of expressing at least one α-Gal A protein in a cell, the method comprising administering the expression construct of any one of claims 1-10 to the cell such that the α-Gal A protein is expressed in the cell.

15. A genetically modified cell comprising the expression construct of any one of claims 1-10.

16. The genetically modified cell of claim 14, wherein the cell is made by the method of claim 14.

17. The genetically modified cell of claim 15 or 16, wherein (a) the cell is a stem cell or a precursor cell; or (b) the cell is a liver or muscle cell.

18. Use of the expression construct of any one of claims 1-10, the composition of any one of claims 11-13, or the cell of any one of claims 15-17, for the manufacture of a medicament for use in preventing, inhibiting, or treating Fabry disease or one or more symptoms associated with Fabry disease.

19. The use of claim 18, wherein the symptoms comprise one or more of Gb3 levels above normal, lyso-Gb3 levels above normal, renal disease, cardiac disease, acroparesthesia, angiokeratomas, GI tract pain, corneal and lenticular opacities, or cerebrovascular disease.

20~~13~~. The ~~method~~ use of ~~any one of~~ claim~~s~~ ~~5-12~~ 18 or 19, wherein the expression ~~vector~~ construct is administered to ~~the~~ a subject by intravenous infusion.

21~~14~~. The ~~method~~ use of claim 20~~any one of claims 5-13~~, wherein only one dose of the expression construct is administered to the subject.

22~~15~~. The ~~method~~ use of claim 20 or 21~~any one of claims 5-14~~, wherein the subject is administered an immunosuppressant prior to and/or during administration of the expression construct.

23~~16~~. The ~~method~~ use of claim 22~~15~~, wherein the immunosuppressant comprises prednisone.

24~~17~~. The ~~method~~ use of any one of claims 20-23~~1-16~~, wherein expression of the at least one ~~α-galactosidase A (~~α-Gal A~~)~~ protein is sustained for at least 3 months, at least 9 months, or at least 12 months.

25~~18~~. The ~~method~~ use of any one of claims 20-24~~5-17~~, wherein the α-Gal A protein expressed from the transgene decreases the amount of glycospingolipids in the subject by ~~between~~ at least about 2-fold ~~to about 9-fold as compared to untreated subjects~~.

26~~19~~. The ~~method~~ use of any one of claims 20-25 ~~5-18~~, wherein the α-Gal A protein expressed from the transgene decreases the amount of glycospingolipids in the subject by at least about 80%.

27~~20~~. The ~~method~~ use of any one of claims 20-26 ~~5-19~~, wherein the α-Gal A protein expressed from the transgene decreases the amount of glycospingolipids in one or more of the subject's plasma, liver, heart, kidney, or spleen.

2~~8~~1. The ~~method~~ use of any one of claims 20-27 ~~5-20~~, wherein the expression construct manufactured in a HEK293 cell system provides ~~GLA~~ the α-Gal A protein levels in the subject at about 21-fold higher as compared to ~~GLA~~ the α-Gal A protein levels in subjects administered the expression construct manufactured in a Sf9 cell system.

2~~9~~2. The ~~method~~ use of any one of claims 20-28 ~~5-21~~, wherein the α-Gal A protein activity in the ~~a~~ subject is between about 100-fold higher to 1,500-fold higher than physiologial normal/wild type.

3023. The ~~method~~ use of any one of claims 20-29 ~~5-22~~, wherein the α-Gal A protein expressed from the transgene is active in kidneys, liver, and/or heart of the subject.

31. An *in vitro* method of producing an α-Gal A protein for the treatment of Fabry disease, the method comprising expressing the α-Gal A protein in an isolated cell according to the method of claim 14, and isolating the α-Gal A protein produced by the cell.

~~24. The method of any one of claims 1-23, wherein the GLA transgene is maintained extra-chromosomally and not integrated into a genome of the cell.~~

~~25. The method of any one of claims 1-23, further comprising administering one or more nucleases that cleave an endogenous albumin gene in a liver cell in a subject such that the transgene is integrated into and expressed from the albumin gene.~~

~~26. A genetically modified cell comprising an exogenous GLA transgene, made by the method of any one of claims 1-4.~~

~~27. The genetically modified cell of claim 26, wherein the cell is a stem cell or a precursor cell.~~

~~28. The genetically modified cell of claim 27, wherein the cell is a liver or muscle cell.~~

~~29. The genetically modified cell of any one of claims 26-28, wherein the GLA transgene is maintained extra-chromosomally and not integrated into the genome of the cell.~~

~~30. The genetically modified cell of any one of claims 26-28, wherein the GLA transgene is integrated into the genome of the cell.~~

~~31. A method of preventing, inhibiting or treating Fabry disease or one or more symptoms associated with Fabry disease, the method comprising, administering an expression construct to a subject in need thereof, the expression construct comprising a mutated WPRE sequence, optionally a mut6 mutated WRPE sequence, and a GLA transgene encoding at least one α-Gal A protein.~~

~~32. The method of claim 31, wherein the symptoms comprise one or more of Gb3 levels above normal, lyso-Gb3 levels above normal, renal disease, cardiac disease, acroparesthesia, angiokeratomas, GI tract pain, corneal and lenticular opacities, or cerebrovascular disease.~~

~~33. The method of claim 31 or 32, wherein the subject is male and wherein the subject has α-Gal A enzyme activity of less than about 5%.~~

~~34. The method of claim 31, wherein the expression construct comprises a wild-type GLA sequence or a codon optimized GLA sequence.~~

~~35. The method of claim 31, 33, or 34 wherein the expression construct further comprises one or more of the following: an enhancer, a promoter, an intron, a sequence encoding a signal peptide and/or a polyadenylation signal, wherein the mutated WPRE sequence, optionally the mut6 mutated WRPE sequence, and the GLA transgene encoding at least one α-Gal A protein is located between the signal peptide and the sequence encoding the polyadenylation signal.~~

~~36. The method of claim 31, wherein the expression construct is administered in a pharmaceutically acceptable carrier.~~

~~37. The method of claim 36, wherein the pharmaceutically acceptable carrier comprises phosphate buffered saline containing $CaCl_2$, Mg Cl_2, NaCl, sucrose and Kolliphor (Poloxamer) P 188.~~

~~38. The method of claim 31, wherein the expression construct sequence comprises the sequence as shown in Table 1 and wherein the expression construct is delivered to cells of the subject by an AAV viral vector.~~

~~39. The method of claim 38, wherein the AAV viral vector serotype is AAV2/6.~~

~~40. The method of any one of claims 31-39, wherein the expression construct is administered to the subject at a dose of between about 5.0E+12 and 1.0E+14 vector genomes per kilogram (vg/kg).~~

~~41. The method of any one of claims 31-40, wherein the expression construct is administered to the liver of the subject.~~

~~42. The method of any one of claims 31-41, wherein the expression vector is administered to the subject by intravenous infusion.~~

~~43. The method of any one of claims 31-42, wherein only one dose of the expression construct is administered to the subject.~~

~~44. The method of any one of claims 31-43, wherein the subject is administered an immunosuppressant prior to and/or during administration of the expression construct.~~

~~45. The method of claim 44, wherein the immunosuppressant comprises prednisone.~~

~~46. The method of any one of claims 31-45, wherein expression of the at least one α galactosidase A (α-Gal A) protein is sustained for at least 3 months, at least 9 months, or at least 12 months.~~

~~47. The method of any one of claims 31-46, wherein the α-Gal A protein expressed from the transgene decreases the amount of glycospingolipids in the subject by between at least about 3-fold to about 9-fold as compared to untreated subjects.~~

~~48. The method of any one of claims 31-46, wherein the α-Gal A protein expressed from the transgene decreases the amount of glycospingolipids in the subject by at least about 80%.~~

~~49. The method of any one of claims 31-48, wherein the α-Gal A protein expressed from the transgene decreases the amount of glycospingolipids in one or more of the subject's plasma, liver, heart, kidney, or spleen.~~

~~50. The method of any one of claims 31-49, wherein the expression construct is manufactured in a HEK293 cell system and wherein the GLA levels in the subject are 21-fold higher as compared to GLA levels in subjects administered the expression construct manufactured in a Sf9 cell system.~~

~~51. The method of any one of claims 31-50, wherein α-Gal A protein activity in a subject is between about 100-fold higher to 1,500-fold higher than physiological normal.~~

~~52. The method of any one of claims 31-51, wherein the α-Gal A protein expressed from the transgene is active in kidneys, liver and heart of the subject.~~

~~53. The method of any one of claims 31-52, wherein the GLA transgene is maintained extra-chromosomally and not integrated into a genome of the subject's cell.~~

~~54. The method of any one of claims 31-52, further comprising administering one or more nucleases that cleave an endogenous albumin gene in a liver cell in a subject such that the transgene is integrated into and expressed from the albumin gene.~~

~~55. A composition comprising an expression construct, the expression construct comprising a mutated WPRE sequence, optionally a mut6 mutated WRPE sequence, and a GLA transgene encoding the at least one α-Gal A protein for the treatment of Fabry's disease.~~

~~56. The composition of claim 55, further comprising a pharmaceutically acceptable carrier.~~

~~57. The composition of claim 56, wherein the pharmaceutically acceptable carrier comprises $CaCl_2$, Mg Cl_2, NaCl, sucrose and Kolliphor (Poloxamer) P 188.~~

~~58. The composition of claim 55, wherein the expression construct comprises a wild-type GLA sequence or a codon optimized GLA sequence.~~

~~59. The composition of any one of claims 55-58, wherein the expression construct further comprises one or more of the following: an enhancer, a promoter, an intron, a sequence encoding a signal peptide and/or a polyadenylation signal, wherein the mutated WPRE sequence, optionally the mut6 mutated WRPE sequence, and the GLA transgene encoding at least one α-Gal A protein is located between the signal peptide and the sequence encoding the polyadenylation signal.~~

~~60. The composition of any one of claims 55-59, wherein the expression construct sequence comprises the sequence as shown in Table 1 and wherein the expression construct is delivered to a cell by an AAV viral vector.~~

~~61. The composition of any one of claims 55-60, wherein the AAV viral vector serotype is AAV2/6.~~

~~62. The composition of claim 60 or 61, wherein the expression construct comprises between about 5.0E+12 and 1.0E+14 vector genomes per subject kilogram (vg/kg).~~

~~63. The composition of claim 59, wherein the expression construct comprises the sequence of SEQ ID No: 9.~~

~~64. A method of producing an α-Gal A protein for the treatment of Fabry disease, the method comprising expressing the α-Gal A protein in an isolated cell according to the method of any one of claims 1-4, and isolating the α-Gal A protein produced by the cell.~~

~~65. A delivery vector comprising a mutated WRPE sequence, optionally a mut6 WPRE sequence and a GLA transgene for use in the method of claim 1.~~

~~66. The vector of claim 65, wherein the delivery vector is a viral vector or a lipid nanoparticle (LNP).~~

~~67. The vector of claim 66, wherein the viral vector comprises an AAV2/6 and wherein the viral vector delivers the expression construct to at least 50%, at least 60%, at least 70%, or at least 80% of cells.~~

~~68. Use of an expression construct, an AAV vector and/or genetically modified cell of any of the preceding claims for the treatment of Fabry's disease.~~

~~69. The method of claim 3, wherein the enhancer comprises SEQ ID No: 2, the promotor comprises SEQ ID No: 3, the intron comprises SEQ ID No: 4, the GLA transgene comprises SEQ ID No: 5, the mutated WPRE sequence comprises SEQ ID No: 6, and the polyadenylation signal comprises SEQ ID No. 7.~~

~~70. The composition of claim 59, wherein the enhancer comprises SEQ ID No: 2, the promotor comprises SEQ ID No: 3, the intron comprises SEQ ID No: 4, the GLA transgene comprises SEQ ID No: 5, the mutated WPRE sequence comprises SEQ ID No: 6, and the polyadenylation signal comprises SEQ ID No. 7.~~