[0008] Gene therapy for patients with Hemophilia A or B, involving the introduction of plasmid and other vectors (*e.g*., AAV) encoding a functional FVIII or F.IX proteins have been described. (See, *e.g*., U.S. Patent Nos. 6,936,243; 7,238,346 and 6,200,560; Shi *et al*. (2007) *J Thromb Haemost*. (2):352-61; Lee *et al*. (2004) *Pharm. Res*. 7:1229-1232; Graham *et al*. (2008) *Genet Vaccines Ther*. 3:6-9; Manno *et al*. (2003) *Blood* 101(8): 2963-72; Manno *et al.* (2006) *Nature Medicine* 12(3): 342-7; Nathwani *et al*. (2011) *Mol Ther* 19(5): 876-85; Nathwani *et al*. (2011); *N Engl J Med*. 365(25): 2357-65 and McIntosh *et al*. (2013) *Blood* 121(17):3335-44). However, in these protocols, the formation of inhibitory anti-factor VIII or IX (anti-FVIII or anti-F.IX) antibodies, and antibodies against the delivery vehicle remains a major complication of FVIII and F.IX replacement-based treatment for hemophilia. See, *e.g*., Scott & Lozier (2012) *Br J Haematol.* 156(3):295-302.

[0009] However, there remains a need for liver-specific polynucleotides (expression constructs and transcription modules) that drive expression of one or more transgenes (including transgenes encoding one or more proteins lacking in a hemophilia) in liver cells at high levels.

SUMMARY

[0010] The present disclosure describes compositions and methods for expressing a transgene in a liver cell. The transgene may be expressed extra-chromosomally (episomally) or may be integrated into the genome of the liver cell (*e.g.,* via nuclease-mediated targeted integration, for example into an albumin locus). In some examples, the transgene encodes a protein involved in the clotting cascade. In preferred examples, the transgene encodes a FVIII polypeptide. The compositions and methods described herein result in high levels of protein production both *in vitro* and *in vivo*, including at levels sufficient to show clinically relevant (therapeutic) effects *in vivo*.

[0011] In one aspect, described herein is a polynucleotide expression construct comprising at least one spacer sequence comprising an insulator sequence, a liver-specific enhancer sequence (*e.g.,* a wild-type or mutated Serpin 1 enhancer sequence), a promoter sequence (*e.g.,* a wild-type or mutated transthyretin (TTR) promoter) and a transgene (*e.g.,* a nuclease and/or a therapeutic protein such as a protein lacking and/or deficient in a hemophilia or a lysosomal storage disease). In certain examples, the polynucleotide expression construct further comprises an intron sequence (*e.g.,* a

wild-type or mutated minute virus of mice (MVM) intron sequence). In certain examples, the polynucleotide expression cassette comprises two spacer sequences flanking the liver-specific enhancer sequence, the promoter sequence, the intron sequence and the transgene and optionally, a polyadenylation signal. Any insulator sequence(s) can be used, including but not limited to any wild-type or mutated insulator sequence (*e.g.,* one or more of SEQ ID NO:28, 29, 30 and/or 38 in any combination). In certain examples, the polynucleotide expression construct comprises a polynucleotide designated CRMSBS1 (SEQ ID NO:37) or CRMSBS2 (SEQ ID NO:34).

[0012] In another example, described herein is a polynucleotide expression construct comprising a liver-specific enhancer sequence (*e.g.,* a wild-type or mutated Serpin 1 enhancer sequence), a promoter sequence (*e.g.,* a wild-type or mutated transthyretin (TTR) promoter), an intron sequence as shown in any one of SEQ ID NO:15, 16 or 17 and a transgene (*e.g.,* a nuclease and/or a therapeutic protein such as a protein lacking and/or deficient in a hemophilia or a lysosomal storage disease). In certain examples, the polynucleotide expression cassette further comprises at least one spacer sequence comprising an insulator sequence and/or a polyadenylation signal.

[0012a] In another aspect, described herein is a polynucleotide expression construct comprising a liver-specific enhancer sequence, a promoter sequence, an intron sequence as shown in nucleotides 340-432 of SEQ ID NO:37 or any one of SEQ ID NO:15, 16 or 17 and a transgene.

[0013] In yet another example, described herein is a polynucleotide expression construct comprising a liver-specific enhancer sequence having mutations at positions 1, 5, 14, 32 and/or 39 of any of SEQ ID NOs:1-13 (*e.g.,* as shown in SEQ ID NO:35 or 36), a promoter sequence (*e.g.,* a wild-type or mutated transthyretin (TTR) promoter), and a transgene (*e.g.,* a nuclease and/or a therapeutic protein such as a protein lacking and/or deficient in a hemophilia or a lysosomal storage disease). In certain examples, the polynucleotide expression constructs further comprises an intron sequence (*e.g.,* a wild-type or mutated minute virus of mice (MVM) intron sequence) and/or at least one spacer sequence comprising an insulator sequence and/or a polyadenylation signal.

[0014] In other aspects, an AAV vector comprising any of the polynucleotide expression constructs described herein is provided (*e.g.,* in which polynucleotide

expression construct is between the 5' and 3' inverted terminal repeats (ITRs) of the AAV vector).

[0015] In yet other examples, provided herein are pharmaceutical compositions comprising one or more AAV vectors and/or one or more polynucleotide expression constructs as described herein.

[0015a] In another aspect, provided herein is a pharmaceutical composition comprising an AAV vector as described herein.

[0016] Also provided are methods for providing a protein to a subject in need thereof, the method comprising administering to the liver of the subject a polynucleotide expression construct, an AAV vector or a pharmaceutical composition as described herein to the subject, wherein the transgene encodes the protein and the protein is produced in the subject. In certain examples, the transgene is integrated into the genome of a liver cell in the subject. Optionally, the methods further comprise administering one or more nucleases to the subject, wherein the nuclease cleave an endogenous albumin gene and the transgene is integrated into the endogenous albumin gene. Methods of genetically modifying a cell to include a transgene (*e.g.,* that produces a protein) are also provided, including methods introducing the transgene into the cell (episomally or integrated including nuclease-mediated targeted integration). Also provided are methods of inducing tolerance in a mammal to a therapeutic protein, the method comprising genetically modifying a cell (*e.g.,* a cell that has been modified to produce a protein as described herein) in a subject as described herein and treating the one or more steroids and/or B-cell inhibitors such that the mammal becomes tolerized to the therapeutic protein.

[0017] In one example, described herein is a polynucleotide expression construct comprising an enhancer sequence (*e.g.,* a wild-type or mutated Serpin1 enhancer), a promoter sequence (*e.g.,* a transthyretin minimal promoter (TTRm) promoter), and the transgene and, optionally, a polyadenylation sequence (*e.g.,* a synthetic polyadenylation sequence (SPA) and/or a signal peptide (SP). In certain examples, the expression construct further comprises an intron sequence (*e.g.,* wild-type MVM or a mutated MVM sequence and/or chimeric intron). In certain examples, the expression constructs comprise in 5' to 3' orientation, an enhancer sequence, a promoter sequence, an intronic sequence, a transgene (optionally comprising a signal peptide), and a polyadenylation signal.

[0018] The expression cassette may be included in any viral or non-viral vector, including but not limited to plasmid vectors, adenovirus vector, retroviral vectors and adeno associated vector (AAV). In a preferred example, the expression construct is carried on an AAV construct and further comprises 5' and 3' ITRs flanking the expression constructs as described herein. Optionally, insulator (spacer) molecules are also included between one or more of the components of the expression construct, for example, between the 5' ITR and the enhancer and/or between the polyadenylation signal and the 3' ITR. In some examples, the insulator (spacer) regions comprise homology arms to facilitate targeted integration. In certain examples, the construct is a construct as shown in any of Figures 1, 2, 4, 6, 7, 10, 19, 20 or 25. Two exemplary constructs are shown in SEQ ID NO:34 and in SEQ ID NO:37. It will be apparent that the individual components (promoter, insulator(s), enhancer, transgene) can be combined in any combination with other components as described herein.

[0019] In any of the polynucleotides describes herein, the enhancer may be derived from a Serpin-1 enhancer. In certain examples, the enhancer is a wild-type sequence. In other examples, the enhancer comprises one or more modifications as compared to wild-type, for example a Serpin1 enhancer containing one or more nucleotide modifications as shown in Figure 5, modifications of nucleotides at one or more of residues 1, 5, 14, 32 and/or 39 of the sequence shown in any of SEQ ID NOs:1-13. In certain examples, the Serpin1 enhancer comprises modifications at positions 1, 5, 14 and 32 of any of SEQ ID NOs:1-13 while in other examples, the Serpin1 enhancer comprises modification at positions 1, 14, 32 and 39 or any of SEQ ID NOs:1-13. Exemplary enhancer sequences are shown in SEQ ID NO:35 and 36. In some examples, the polynucleotides described herein comprise 1, 2, 3, 4, 5 or more enhancer elements. In certain examples, the 1, 2, 3, 4, 5 or more enhancer elements are identical wherein other examples, more than one type of enhancer element is used.

[0020] Any of the polynucleotides described herein may further optionally comprise an intronic sequence. In certain examples, the expression construct include a chimeric intron sequence, for example as shown in the bottom panels of Figures 6 and 7. The T-chimeric intron is a truncated version of the chimeric intron in pCI-neo (GenBank U47120). The chimeric intron in pCI-neo is the 5' splice donor site from the human β-globin gene, and the branchpoint and 3' acceptor site of an

immunoglobulin gene heavy chain variable region. The T-chimeric intron contains a 45 bp deletion between the 5' splice donor and the branchpoint. In yet other examples, the expression constructs include a mutated MVM intron sequence (*e.g.,* one or more of the mutations shown in Figures 12, 13 and 14 (SEQ ID NOs:15-17). Alternatively, the expression constructs as described herein may lack an intronic sequence, for example as shown in the constructs depicted in the middle panels of Figures 6, 7, 19 and 20.

[0021] The expression constructs of the disclosure also may include optimized insulator sequences between the AAV ITRs and the expression cassette. In certain examples, the expression construct comprises insulator (spacer) regions (*e.g*. Ins1 and Ins3, SEQ ID NOs 15 or 28 and SEQ ID NOs: 17 or 30, (respectively), and Ins2 (SEQ ID NOs:16 or 29). In certain examples, the expression construct comprises Ins4 (SEQ ID NO:38). Any of the insulator sequences may be used in either the 5' or 3' location, and any combination of the insulator sequences may be used within an expression construct. Particularly preferred are combinations of Ins1 at the 5' location and Ins3 at the 3' location.

[0022] The expression constructs as described herein also include and express one or more transgenes. Any transgene(s) can be expressed using the polynucleotides described herein, including, but not limited to, transgenes encoding functional versions of proteins lacking or deficient in any genetic disease, including but not limited to, lysosomal storage disorders (*e.g.,* Gaucher's, Fabry's, Hunter's, Hurler's, Neimann- Pick's, Phenylketonuria (PKU) etc.), metabolic disorders, and/or blood disorders such as hemophilias and hemoglobinopathies, etc. *See, e.g.,* U.S. Publication No. 20140017212 and 20140093913; U.S. Patent Nos. 9,255,250 and 9,175,280. Non-limiting examples of proteins that may be expressed as described herein include fibrinogen, prothrombin, tissue factor, Factor V, Factor VII, Factor VIIII, Factor IX, Factor X, Factor XI, Factor XII (Hageman factor), Factor XIII (fibrin-stabilizing factor), von Willebrand factor, prekallikrein, high molecular weight kininogen (Fitzgerald factor), fibronectin, antithrombin III, heparin cofactor II, protein C, protein S, protein Z, protein Z-related protease inhibitor, plasminogen, alpha 2-antiplasmin, tissue plasminogen activator, urokinase, plasminogen activator inhibitor-1, plasminogen activator inhibitor-2, glucocerebrosidase (GBA), α-galactosidase A (GLA), iduronate sulfatase (IDS), iduronidase (IDUA), acid sphingomyelinase (SMPD1), MMAA, MMAB, MMACHC, MMADHC (C2orf25),

MTRR, LMBRD1, MTR, propionyl-CoA carboxylase (PCC) (PCCA and/or PCCB subunits), a glucose-6-phosphate transporter (G6PT) protein or glucose-6-phosphatase (G6Pase), an LDL receptor (LDLR), ApoB, LDLRAP-1, a PCSK9, a mitochondrial protein such as NAGS (N-acetylglutamate synthetase), CPS1 (carbamoyl phosphate synthetase I), and OTC (ornithine transcarbamylase), ASS (argininosuccinic acid synthetase), ASL (argininosuccinase acid lyase) and/or ARG1 (arginase), and/or a solute carrier family 25 (SLC25A13, an aspartate/glutamate carrier) protein, a UGT1A1 or UDP glucuronsyltransferase polypeptide A1, a fumarylacetoacetate hydrolyase (FAH), an alanine-glyoxylate aminotransferase (AGXT) protein, a glyoxylate reductase/hydroxypyruvate reductase (GRHPR) protein, a transthyretin gene (TTR) protein, an ATP7B protein, a phenylalanine hydroxylase (PAH) protein, a lipoprotein lyase (LPL) protein, an engineered nuclease, an engineered transcription factor and/or an engineered single chain variable fragment antibody (diabody, camelid, etc.). In one preferred example, the transgene encodes a FVIII polypeptide. In some examples, the FVIII polypeptide comprises a deletion of the B domain.

[0023] In some examples, the one or more transgenes include sequences encoding engineered nucleases (*e.g*. ZFNs, TALENs, TtAgo and CRISPR/Cas systems). In other examples, the transgenes include sequences encoding engineered transcription factors (*e.g*. ZFP-TFs, TALE-TFs, CRISPR/Cas-TF systems). The transgenes may also include sequences encoding a single chain antibody specific for a target of interest. In addition, the transgene may include sequences encoding a structural RNA (*e.g*. RNAi, shRNA, miRNA).

[0024] In certain examples, the polynucleotides as described herein are introduced into a cell such that they are maintained episomally while driving expression of the transgene. In other examples, the expression constructs are randomly integrated into the genome of the cell into which they are introduced. In further examples the expression constructs driving transgene expression are integrated into a genome by nuclease-mediated targeted integration.

[0025] In further xamples, described herein are methods for expressing one or more transgenes in a liver cell, the methods comprising introducing one or more expression constructs as described herein into the cell such that the transgene is expressed in the cell. In certain examples, the expression construct is carried on a viral or non-viral vector, preferably an AAV vector (for example AAV2 or AAV2/6).

[0026] In another example, provided herein is a method of expressing one or more transgenes in a live animal, the methods comprising administering one or more expression cassettes as described herein to the live animal. In certain examples, the expression cassettes are administered to the liver of the live animal. In certain examples, the expression construct is carried on a viral or non-viral vector, preferably an AAV vector (for example AAV2, AAV2/6 or AAV2/8).

[0027] In some examples, provided herein are methods and compositions to express therapeutically relevant levels of one or more therapeutic proteins from one or more transgenes. In certain examples, expression of a transgene construct encoding a replacement protein results in 1% of normal levels of the protein produced, while in others, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 30%, 50%, 80%, 100%, 150%, 200%, or more of normal levels of the protein are produced. In some preferred examples, the transgene encodes a FVIII protein and a therapeutically relevant amount of the protein is produced. In some examples, as a result of the use of the methods and compositions of the disclosure, a human patient has an increased amount of a therapeutic protein in their blood that results in a decrease in clinical symptoms. In certain examples, the production of the therapeutic protein by the methods and compositions described herein in a human patient results in a decreased clotting time following injury as compared to a patient that has not been treated or compared to the patient prior to treatment. In some examples, a human patient treated with the methods and compositions of the disclosure requires a decreased amount of replacement therapy than a patient who has not been treated or compared to the patient prior to treatment.

[0028] In some examples, the method and compositions of the disclosure as described herein can be used to induce tolerance in a mammal to a therapeutic protein such that the levels of the therapeutic protein encoded by the transgene remain at therapeutically relevant levels following a transient rise in anti-therapeutic protein antibodies. Thus, provided herein is a method of inducing tolerance to a therapeutic protein in a subject, the method comprising genetically modifying a cell in a subject using the method as described herein (*e.g.*, so that the cell produces the therapeutic protein), optionally treating the subject with additional compositions (*e.g.*, steroids and/or B-cell inhibitors) such that the animal becomes tolerized to the therapeutic protein. In some examples, insertion (integration) of the therapeutic protein into the recipient cells is done at the same time as treatment with an immune-inhibitory steroid or B-cell inhibitor, whereas in other instances, no immunomodulatory substances are

administered to the animal. In some instances, the immunomodulatory agent is administered only if anti-therapeutic protein antibodies are generated. In further instances, the immunomodulatory agent is discontinued after a period of time.

[0029] In another example, pharmaceutical compositions comprising one or more of the cells, expression constructs and/or optional nucleases described herein are provided.

[0030] In certain examples, described herein are methods and systems for targeted integration of a liver-specific expression cassette. The methods and systems comprise administering one or more expression cassettes as described herein and administering one or more nucleases specific for a target gene to a cell. Following nuclease-mediated cleavage of the target gene, the expression cassette is integrated into the gene via homology-dependent or homology-independent mechanisms. In certain examples, the target gene is an endogenous albumin gene.

[0031] For nuclease-mediated targeted integration of the expression constructs of the present disclosure, any nuclease can be used, including but not limited to, one or more zinc finger nucleases (ZFNs), TALENs, CRISPR/Cas nucleases and/or TtAgo nucleases, such that the expression construct is integrated into the region (gene) cleaved by the nuclease(s). In certain examples, one or more pairs of nucleases are employed. The nucleases may be introduced in mRNA form or may be administered to the cell using non-viral or viral vectors. In some examples, the nuclease polynucleotides may be delivered by lentivirus or by non-integrating lentivirus. In other examples, the expression cassette may be delivered by AAV and/or DNA oligos.

[0032] In another example, provided herein are methods for providing one or more functional proteins lacking or deficient in a mammal, or in a primate, such as a human primate, such as a human patient with a disease (*e.g.,* a metabolic disease, a lysosomal storage disease (LSD), a hemoglobinopathy and/or a hemophilia), for example for treating the disease by supplying the protein(s) lacking or deficient in the subject. In another example, provided herein are methods for providing a functional protein for treating a disorder in which the protein is lacking, deficient or aberrantly expressed. In further examples, the methods comprise administering an expression cassette encoding a therapeutic protein useful for preventing or treating a disorder. In a further example, methods are described herein for providing a therapeutic protein for treating a disorder where the therapeutic protein is a single chain antibody. In

certain examples, the methods comprise administering an expression cassette (*e.g.,* AAV vector) as described herein to the liver of a subject in need thereof. In other examples, the method comprises administering a modified cell (expressing a functional version of a protein that is aberrantly expressed in a subject from an expression cassette as described) to the subject. Thus, an isolated cell may be introduced into the subject (*ex vivo* cell therapy) or a cell may be modified when it is part of the subject (*in vivo*).

[0033] In any of the compositions and methods described, expression cassettes and/or nucleases may be carried on an AAV vector, including but not limited to AAV1, AAV3, AAV4, AAV5, AAV6, AAV8, AAV9 and AAVrh10 or pseudotyped AAV such as AAV2/8, AAV8.2, AAV2/5 and AAV2/6 and the like. In certain examples, the polynucleotides (expression constructs and/or nucleases) are delivered using the same AAV vector types. In other examples, the polynucleotides are delivered using different AAV vector types. The polynucleotides may be delivered using one or more vectors. In certain examples, the polynucleotides are delivered via intravenous (*e.g.,* intra-portal vein) administration into the liver of an intact animal. In other examples, the polynucleotides are delivered via intravenous administration in a peripheral vein.

[0034] In any of the compositions and methods described herein, the protein encoded by the transgene may comprise a F8 protein, for example a B-Domain Deleted Factor VIII (BDD-F8). In other examples, the protein encoded by the transgene comprises a Factor IX protein. In other examples, the protein encoded by the transgene comprises a Factor VII protein. In other examples, the protein encoded by the transgene comprises a Factor X protein. In some examples, the protein encoded by the transgene comprises a glucocerebrosidase. In other examples, the protein encoded by the transgene comprises an α galactosidase. In further examples, the protein encoded by the transgene comprises an iduronate-2-sulfatase. In some examples, the protein encoded by the transgene comprises an alpha-L iduronidase. In further examples, the protein encoded by the transgene comprises sphingomyelin phosphodiesterase. In some examples, the transgene encodes a single chain antibody. In other examples, the transgene encodes a structural RNA. In any of the compositions or methods described herein, the transgene also comprises a transcriptional regulator while in others, it does not and transcription is regulated by an endogenous regulator. In another example, the methods of the disclosure comprise

a composition for therapeutic treatment of a subject in need thereof. In some examples, the composition comprises engineered stem cells comprising a safe harbor specific nuclease, and a transgene encoding Factor VII, F8, F.IX, Factor X, GBA, GLA, IDS, IDUA, single chain antibody and/or SMPD1 protein or a functional fragment and/or truncation thereof. In other examples, the composition comprises engineered stem cells that have been modified and express a transgene encoding Factor VII, F8, F.IX, Factor X, GBA, GLA, IDS, IDUA, single chain antibody and/or SMPD1 protein or a functional fragment and/or truncation thereof.

[0035] The methods described herein can be practiced *in vitro, ex vivo* or *in vivo.* In certain examples, the compositions are introduced into a live, intact mammal. The mammal may be at any stage of development at the time of delivery, *e.g.*, embryonic, fetal, neonatal, infantile, juvenile or adult. Additionally, targeted cells may be healthy or diseased. In certain examples, one or more of the compositions are delivered intravenously (*e.g.,* to the liver via the intraportal vein, for example tail vein injection), intra-arterially, intraperitoneally, intramuscularly, into liver parenchyma (*e.g.,* via injection), into the hepatic artery (*e.g.,* via injection), and/or through the biliary tree (*e.g.,* via injection).

[0036] For targeting the compositions to a particular type of cell, *e.g.*, platelets, fibroblasts, hepatocytes, etc., one or more of the administered compositions may be associated with a homing agent that binds specifically to a surface receptor of the cell. For example, the vector may be conjugated to a ligand (*e.g.*, galactose) for which certain hepatic system cells have receptors. The conjugation may be covalent, *e.g.*, a crosslinking agent such as glutaraldehyde, or noncovalent, *e.g.,* the binding of an avidinated ligand to a biotinylated vector. Another form of covalent conjugation is provided by engineering the AAV helper plasmid used to prepare the vector stock so that one or more of the encoded coat proteins is a hybrid of a native AAV coat protein and a peptide or protein ligand, such that the ligand is exposed on the surface of the viral particle.

[0037] A kit, comprising one or more of the expression constructs, AAV vectors, cell and/or pharmaceutical compositions described herein, is also provided. The kit may further comprise nucleic acids encoding nucleases, (*e.g.* RNA molecules encoding ZFNs, TALENs or Cas and modified Cas proteins, and guide RNAs), or

aliquots of the nuclease proteins, cells, instructions for performing the methods of the disclosure, and the like.

[0037a] In another aspect, provided herein are kits comprising a polynucleotide expression cassette as disclosed herein, an AAV vector as disclosed herein and/or a pharmaceutical composition as disclosed herein.

[0037b] In another aspect, provided herein are uses of an AAV vector as described herein or a pharmaceutical composition as described herein in the manufacture of a medicament for providing a protein to a subject in need thereof, wherein the medicament is to be administered to the liver of the subject, and wherein the transgene encodes the protein and the protein is produced in the subject.

[0037c] In another aspect, provided herein is a polynucleotide expression construct comprising SEQ ID NO: 34.

[0037d] In another aspect, provided herein is a polynucleotide expression construct comprising SEQ ID NO: 37.

[0038] These and other examples will be readily apparent to the skilled artisan in light of disclosure as a whole.

CLAIMS

What is claimed is:

1. A polynucleotide expression construct comprising at least one spacer sequence comprising an insulator sequence, a liver-specific enhancer sequence, a promoter sequence and a transgene.

2. The polynucleotide expression construct of claim 1, further comprising an intron sequence, wherein the enhancer sequence comprises a wild-type or mutated Serpin 1 enhancer; and/or the promoter sequences comprises a wild-type or mutated transthyretin (TTR) promoter; and/or the intron sequence comprises a wild-type or mutated minute virus of mice (MVM) intron sequence.

3. The polynucleotide expression cassette of claim 1 or claim 2, wherein the insulator sequence is any one of SEQ ID NO:28, 29 and/or 30.

4. The polynucleotide expression cassette of any of claims 2 to 3, comprising two spacer sequences flanking the liver-specific enhancer sequence, the promoter sequence, the intron sequence and the transgene.

5. The polynucleotide expression cassette of claim 4, wherein the insulator sequences are SEQ ID NO:28, 29, 30 and/or 38.

6. The polynucleotide expression cassette of any of claims 1 to 5, wherein the transgene encodes a protein lacking or deficient in a hemophilia or a lysosomal storage disease.

7. The polynucleotide expression cassette of claim 6, wherein the transgene further encodes one or more nucleases.

8. A polynucleotide expression construct comprising a liver-specific enhancer sequence, a promoter sequence, an intron sequence as shown in nucleotides 340-432 of SEQ ID NO:37 or any one of SEQ ID NO:15, 16 or 17 and a transgene.

9. The polynucleotide expression construct of claim 8, wherein the enhancer sequence comprises a wild-type or mutated Serpin 1 enhancer; and/or the promoter sequences comprise a wild-type or mutated the transthyretin (TTR) promoter.

10. The polynucleotide expression cassette of claim 8 or claim 9, further comprising at least one spacer sequence comprising an insulator sequence and/or a polyadenylation signal.

11. The polynucleotide expression cassette of any of claims 8 to 10, wherein the transgene encodes a protein lacking or deficient in a hemophilia or a lysosomal storage disease.

12. The polynucleotide expression cassette of any of claims 8 to 11, wherein the transgene further encodes one or more nucleases.

13. An AAV vector comprising the polynucleotide expression construct of any of claims 1-12, wherein the polynucleotide expression construct is between the 5' and 3' inverted terminal repeats (ITRs) of the AAV vector.

14. The AAV vector of claim 13, wherein the AAV vector comprises SEQ ID NO:34 or SEQ ID NO:37.

15. A pharmaceutical composition comprising an AAV vector according to claim 13 or 49.

16. Use of an AAV vector according to claim 13 or 14 or a pharmaceutical composition according to claim 20 in the manufacture of a medicament for providing a protein to a subject in need thereof, wherein the medicament is to be administered to the liver of the subject, and wherein the transgene encodes the protein and the protein is produced in the subject.

17. The use of claim 16, wherein the transgene is integrated into the genome of a liver cell.

18. The use of claim 16 or 17, wherein one or more nucleases is to be further administered to the subject, wherein the nuclease cleave an endogenous albumin gene and the transgene is integrated into the endogenous albumin gene.

19. The use of any of claims 16 to 18, wherein the subject is to be further treated with one or more steroids and/or B-cell inhibitors to induce tolerance in the subject to a therapeutic protein.

20. A kit comprising a polynucleotide expression cassette according to any of claims 1 to 12, an AAV vector according to claim 13 or claim 14 and/or a pharmaceutical composition according to claim 15.

21. A polynucleotide expression construct comprising SEQ ID NO: 34.

22. A polynucleotide expression construct comprising SEQ ID NO: 37.