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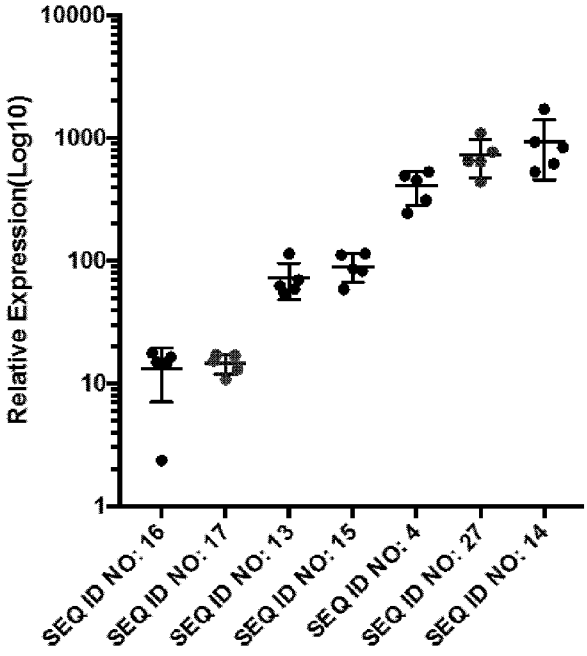
(54) Title: HIGH ACTIVITY REGULATORY ELEMENTS

(57) Abstract: Provided herein are compositions and methods for driving high expression of a transgene. Compositions and methods for driving high expression of a transgene comprising one or more human-derived regulatory elements, which, when operably linked to a transgene, can result in high expression of the transgene in one or more cell types or tissues.

FIG. 13



WO 2018/213786 A1

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HIGH ACTIVITY REGULATORY ELEMENTS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/508,968, filed on May 19, 2017, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 17, 2018, is named 46482-707_601_SL.txt and is 16,836 bytes in size.

BACKGROUND OF THE DISCLOSURE

[0003] Genetic engineering has enormous potential for treatment of disease, and production of proteins either for therapeutic or other uses. Instead of relying on drugs or surgery, patients, especially those with underlying genetic factors, can be treated by directly targeting the underlying cause. Furthermore, by targeting the underlying cause itself, gene therapy has the potential to effectively cure patients. Yet, despite this, clinical applications of gene therapy still require improvement in several aspects. One challenge is achieving high rates of expression of a transgene in one or more target tissues, or high efficiency of gene editing in one or more target tissues. Another challenge is introduced by the packaging limitations (or cloning capacities) of commonly used vectors. There is a need for short regulatory elements which can drive high expression of an operably linked transgene.

SUMMARY OF THE DISCLOSURE

[0004] Described herein are compositions and methods for driving high expression of a transgene. Also described herein are compositions and methods for driving high expression of a transgene comprising one or more human or human-derived regulatory elements (REs) which, when operably linked to a transgene, can facilitate or result in high (or increased) expression of the transgene in one or more target cell types or tissues. In some cases, high or increased transgene expression of any embodiment disclosed herein is determined as compared to a control, e.g., a constitutive promoter, CAG, CMV promoter, super core promoter (SCP), TTR promoter, Proto 1 promoter, UCL-HLP promoter, or CMVe promoter. Other controls that can be used for comparison to determine high or increased transgene expression by a regulatory element disclosed herein include buffer alone, minCMV, EFS,

vector alone, or sequences that do not drive expression. In some embodiments, one or more regulatory elements disclosed herein drive high expression of a transgene in a cell or in vivo, in vitro, and/or ex vivo. In some embodiments, an expression cassette or vector comprising one or more regulatory elements described herein operably linked to a transgene can be adapted for use in any expression system or gene therapy to drive high expression of the transgene in a cell, a subject, or an animal model, or to result in a therapeutically effective level of transgene expression in a cell, a subject, or an animal model. In some embodiments, one or more REs of this disclosure are operably linked to a large transgene (e.g., a transgene whose sequence is more than 1 kb, 1.5kb, 2kb, 2.5kb, 3kb, 3.5kb, 4kb, 4.5kb, 5kb, 5.5kb, 6kb, 6.5kb, 7kb, or 7.5kb) to result in high expression of the transgene in a cell or in vivo. In some cases, such high expression of the transgene in a cell or in vivo is relative to expression of the transgene without said REs, wherein expression of the transgene with the REs is at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 15 fold, at least 20 fold, at least 25 fold, at least 50 fold, at least 100 fold, or more than 100 fold as compared to transgene expression without the REs. In some cases, one or more REs result in such high transgene expression in at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 different cell types. In some cases, one or more REs of this disclosure are operably linked to a transgene for a gene therapy treatment adapted for systemic administration. In some cases, one or more REs of this disclosure are operably linked to a transgene for a gene therapy treatment adapted for expression in the liver or hepatocytes. In some cases, the transgene is a DNA binding protein, e.g., a transcriptional modulator, which modulates an endogenous gene. In some cases, a RE of any embodiment disclosed herein comprises a promoter sequence, an intronic sequence, a 5' UTR sequence, or any combination thereof. In some cases, a RE of any embodiment disclosed herein is a human-derived sequence (or a sequence that has at least 80%, 90%, 95% or 99% sequence identity to a sequence in a human reference genome). In some cases, a RE of any embodiment disclosed herein is less than or equal to 300bp, 250bp, 200bp, 150bp, 140bp, 130bp, 120bp, 110bp, 100bp, 70bp, or 50bp in length. In some cases, an expression construct (e.g., a vector, an AAV, or a viral vector) comprises one or more exogenous RE sequences operably linked to a transgene of this disclosure, wherein each exogenous RE sequence is selected from the sequences listed in **TABLE 1** or SEQ ID NOs: 1-2, 13-17, and 22-41; or wherein each exogenous RE sequence comprises a sequence of **TABLE 1** or SEQ ID NOs: 1-2, 13-17, and 22-41; or wherein each exogenous RE sequence comprises a sequence having

at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity (e.g., local sequence identity) to a sequence of **TABLE 1** or SEQ ID NOs: 1-2, 13-17, and 22-41 (e.g., sequence identity as measured by BLAST). In some cases, an expression cassette (e.g., a vector, an AAV, or a viral vector) comprises one or more RE sequences operably linked to any transgene, wherein each RE sequence is no more than 49bp, 50bp, 56bp, 60bp, 70bp, 80bp, 90bp, 100bp, 110bp, 117bp, 120bp, 130bp, 140bp, 150bp, 160bp, 170bp, 180bp, 190bp, 200bp, 210bp, 220bp, 230bp, 240bp, 250bp, 259bp, 260bp, 265bp, 270bp, 280bp, 290bp, 300bp, 310bp, 320bp, 330bp, 340bp, 350bp, 360bp, 370bp, 380bp, 390bp, or 400bp, and comprises a sequence according to any one of **TABLE 1** or SEQ ID NOs: 1-2, 13-17, and 22-41. In some cases, an expression construct (e.g., a vector, an AAV, or a viral vector) comprises one or more exogenous RE sequences operably linked to a transgene of this disclosure, wherein each RE sequence is no more than 49bp, 50bp, 56bp, 60bp, 70bp, 80bp, 90bp, 100bp, 110bp, 117bp, 120bp, 130bp, 140bp, 150bp, 160bp, 170bp, 180bp, 190bp, 200bp, 210bp, 220bp, 230bp, 240bp, 250bp, 259bp, 260bp, 265bp, 270bp, 280bp, 290bp, 300bp, 310bp, 320bp, 330bp, 340bp, 350bp, 360bp, 370bp, 380bp, 390bp, or 400bp, and comprises a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of **TABLE 1** or SEQ ID NOs: 1-2, 13-17, and 22-41. In some cases, local sequence identity is measured using BLAST. In some cases, percent sequence identity refers to global sequence identity, covering the entire exogenous RE in an expression cassette. In other cases, percent sequence identity refers to local sequence identity, covering a region or part of a region that corresponds to any one of the sequences listed in **TABLE 1**. In some cases, percent sequence identity refers to local sequence identity, covering a region that comprises no more than 49bp, 50bp, 56bp, 60bp, 70bp, 80bp, 90bp, 100bp, 110bp, 117bp, 120bp, 130bp, 140bp, 150bp, 160bp, 170bp, 180bp, 190bp, 200bp, 210bp, 220bp, 230bp, 240bp, 250bp, 259bp, 260bp, 265bp, 270bp, 280bp, 290bp, 300bp, 310bp, 320bp, 330bp, 340bp, 350bp, 360bp, 370bp, 380bp, 390bp, or 400bp of an expression cassette, not including the transgene. As used herein, "relatively short" when describing a RE of the disclosure is preferably a RE sequence that is no more than 45bp, 49bp, 50bp, 56bp, 60bp, 70bp, 80bp, 90bp, 100bp, 110bp, 117bp, 120bp, 130bp, 140bp, 150bp, 160bp, 170bp, 180bp, 190bp, 200bp, 210bp, 220bp, 230bp, 240bp, 250bp, 259bp, 260bp, 265bp, 270bp, 280bp, 290bp, 300bp, 310bp, 320bp, 330bp, 340bp, 350bp, 360bp, 370bp, 380bp, 390bp, or 400bp. Examples of a relatively short RE

include the sequences listed in **TABLE 1**, or SEQ ID NOs: 1-2, 13-17, and 22-41; or sequences having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto and are also no more than 45bp, 49bp, 50bp, 56bp, 60bp, 70bp, 80bp, 90bp, 100bp, 110bp, 117bp, 120bp, 130bp, 140bp, 150bp, 160bp, 170bp, 180bp, 190bp, 200bp, 210bp, 220bp, 230bp, 240bp, 250bp, 259bp, 260bp, 265bp, 270bp, 280bp, 290bp, 300bp, 310bp, 320bp, 330bp, 340bp, 350bp, 360bp, 370bp, 380bp, 390bp, or 400bp.

[0005] In some cases, a RE sequence of any embodiment disclosed herein is located upstream or downstream of a transgene. In some cases, two or more REs of any embodiment disclosed herein are operably linked to a transgene, wherein at least one or more of the REs are located upstream or downstream of the transgene. In some cases, one or more REs of any embodiment of this disclosure are operably linked to a transgene to drive global expression of the transgene, wherein global expression refers to expression in at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 different cell types. In some cases, one or more REs of any embodiment of this disclosure are operably linked to a transgene to drive global expression of the transgene, wherein the construct comprising the REs and the transgene can be delivered systemically, e.g., via oral, intravenous, intramuscular, intraperitoneal, intrathecal, enteral, or parenteral administration. In various embodiments, a RE disclosed herein is human-derived (or a sequence that has at least 80%, 90%, 95% or 99% sequence identity to a sequence in a human reference genome). In some cases, a RE of any embodiment disclosed herein is non-naturally occurring.

[0006] In some instances, an expression vector comprises a human-derived regulatory element having less than or equal to 400bp, 300bp, 250bp, 200bp, 150bp, 140bp, 130bp, 120bp, 110bp, 100bp, 70bp, or 50bp operably linked to a transgene, wherein the regulatory element increases global expression (e.g., expression in at least 2, 3, 4, 5, 6, or more different cell types) of the transgene by at least two fold as compared to a second expression vector without the regulatory element. In some cases, the second expression vector comprises the transgene operably linked to a control promoter, such as a CMV promoter.

[0007] In some cases, a RE of this disclosure comprises a human-derived intronic sequence, e.g., SEQ ID NOs: 1-2. In some cases, an expression cassette of this disclosure comprises one or more of SEQ ID NOs: 1-2 operably linked to a transgene. In some cases, a RE comprising any one or more of SEQ ID NOs: 1-2 is located upstream, downstream, or both upstream and downstream of the transgene.

[0008] In some cases, a RE of this disclosure comprises a human-derived promoter sequence, e.g., SEQ ID NOs: 13-17 and 22-41. In some cases, an expression cassette of this disclosure comprises one or more of SEQ ID NOs: 13-17 and 22-41 operably linked to a transgene. In some cases, a RE comprising any one or more of SEQ ID NOs: 13-17 and 22-41 is located upstream, downstream, or both upstream and downstream of the transgene.

[0009] In some cases, a RE of this disclosure comprises a human-derived intronic sequence and/or a promoter sequence, e.g., two or more of SEQ ID NOs: 1-2, 13-17 and 22-41; or two or more of the sequences listed at **TABLE 1**. In some cases, an expression cassette of this disclosure comprises one or more, two or more, three or more, four or more, or five or more of sequences listed at **TABLE 1** operably linked to a transgene. In some cases, one or more REs listed at **TABLE 1** are located upstream, downstream, or both upstream and downstream of the transgene in an expression cassette. In some cases, one or more REs listed at **TABLE 1** are located upstream of the transgene in an expression cassette disclosed herein.

[0010] In some instances, a RE of any embodiment disclosed herein consists of a sequence selected from **TABLE 1** or SEQ ID NOs: 1-2, 13-17, and 22-41, and any combination thereof. In some cases, two or more REs listed in **TABLE 1** are combined without a linker sequence. In some cases, two or more REs listed in **TABLE 1** are combined using a linker sequence of 1-50bp. In some cases, two or more REs listed in **TABLE 1** are placed in an expression construct such that the 5' start of the RE sequences are less than 2bp, 5bp, 10bp, 20bp, 30bp, 40bp, 50bp, 60bp, 70bp, 80bp, 90bp, 100bp, 200bp, 300bp, 400bp, 500bp, 600bp, 700bp, 800bp, 900bp, 1kb, 1.5kb, 2kb, 2.5kb, 3kb, 3.5kb, 4kb, 4.5kb, 5kb, 5.5kb, 6kb, 6.5kb, 7kb, or 7.5kb apart.

[0011] In some instances, an expression vector comprises a human-derived regulatory element having less than or equal to 300bp, 250bp, 200bp, 150bp, 140bp, 130bp, 120bp, 110bp, 100bp, 70bp, or 50bp operably linked to a transgene, whereby expression of the transgene is higher than that of the same transgene expressed by a control regulatory element, such as a constitutive promoter, CMV promoter, super core promoter, TTR promoter, Proto 1 promoter, UCL-HLP promoter, or CMVe promoter. In some cases, expression of the transgene operably linked to the RE is higher than that of the same transgene linked to a UCL-HLP promoter without the RE.

[0012] In some cases, an expression vector comprises a human-derived regulatory element operably linked to a transgene, wherein a protein encoded by the transgene has (i) a concentration >1.0 IU/mL, >1.5 IU/mL, >2.0 IU/mL, >2.5 IU/mL, or >3.0 IU/mL as measured by an ELISA assay configured to detect the transgene; and/or (ii) > 20%, >25%,

>30%, >35%, >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, or >95%, activity as measured by a Coatest assay. In some cases, such activity or transgene expression is assayed in an animal model, such as a mouse. In some cases, the transgene expression or activity described herein is normalized to a dose of expression cassette, vector, virus, or DNA delivered to cells or a mouse, such as a dose of 16 μg (or 10-20 μg) of an expression vector per mouse.

[0013] In some cases, a vector comprises a human-derived regulatory element having a sequence less than or equal to 300bp, 250bp, 200bp, 150bp, 140bp, 130bp, 120bp, 110bp, 100bp, 70bp, or 50bp in size operably linked to a transgene that is not found in the context with the regulatory element in a cell or in vivo, or is not normally linked to the regulatory element.

[0014] In some cases, the expression cassette or vector described herein comprises a regulatory element that is an intronic sequence, a promoter sequence, an enhancer sequence, or a combination thereof. In some cases, the expression cassette or vector comprises a regulatory element that is SEQ ID NO: 1, 2, 3, 4, 13, 14, 15, 16, 17, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, or 41, or a combination thereof, or a sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto. In some cases, the expression cassette or vector comprises a transgene that is a reporter gene, e.g., luciferase. In some cases, the use of a reporter gene, e.g., luciferase, as the transgene results in global expression that is greater than $1x10^8$ photons/sec as measured in whole mice, or results in increased transgene expression in at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more cell types in an animal, e.g., a mouse. In some cases, the activity or transgene expression of an expression cassette or vector described herein is normalized to a dose of expression cassette, vector, virus, or DNA delivered to a mouse, such as a dose of 12 μg or a dose of 10-20 μg of expression vector per mouse. In some cases, the endogenous version of the transgene disclosed herein is not linked to the regulatory element in a cell or in vivo, or is not normally linked to or associated with the regulatory element in a cell or in vivo. In some cases, global expression of the transgene is at a level greater than expression of the transgene using a vector with a regulatory element selected from the group consisting of: a CMV promoter, a CMVe promoter, a super core promoter, TTR promoter, Proto 1 promoter, and a UCL-HLP promoter. In some cases, expression is detectable in at least 2, 3, 4, 5, 6, 7 or more different cell types in vivo (e.g., in a mouse or in different human cell lines). In some cases, the different cell types are selected from the group consisting of: alveolar cells,

cardiomyocytes, epithelial cells, hepatocytes, intestinal cells, myocytes, neurons, and renal cells. In some cases, a regulatory element as disclosed herein is or comprises an enhancer, a promoter, a 5' UTR sequence, an intronic sequence, or any combination thereof. In some cases, a regulatory element as disclosed herein is or comprises a promoter sequence. In some cases, a regulatory element comprises a promoter that is a CMV promoter, CMV, promoter, super core promoter, TTR promoter, Proto 1 promoter, UCL-HLP promoter, an AAT promoter, a KAR promoter, a EF1α promoter, EFS promoter, or CMVe enhancer/CMV promoter combination, or any combination, variant, or fragment thereof.

[0015] In some cases, an expression vector or cassette disclosed herein further comprises one or more post-transcriptional modification sites. In some cases, the expression vector or cassette comprises a transgene that is Cas9, saCas9, or dCas9. In some cases, the expression vector or cassette comprises a transgene that is a DNA binding protein or a transcriptional modulator, e.g., a transcriptional activator. In some cases, such transcriptional modulator protein acts on an endogenous gene to increase expression of the endogenous gene in a cell or in vivo.

[0016] In other instances, an expression vector comprises a Factor VIII transgene operably linked to a regulatory sequence disclosed herein that is able to drive expression of the Factor VIII to a concentration >1.0 IU/mL as measured by an ELISA assay configured to detect Factor VIII in vivo.

[0017] In some cases, the transgene in any embodiment disclosed herein is a gene associated with a haploinsufficiency. In some cases, such haploinsufficiency is in the liver or hepatocytes. In some cases, the transgene is any one of Factor VIII, Cas9, a hormone, a growth or differentiation factor, insulin, growth hormone, VEGF, neurotrophic factor, fibroblast epithelial factor; cytokine, interleukin, lymphokine, tumor necrosis factor, antibody, immunoglobulin, interferon, chimeric T cell receptor; lipoprotein receptor, cystic fibrosis transmembrane regulator, a gene associated with mucopolysaccharidosis type I, II, III, or IV, beta globin or lipoprotein lipase, or a variant, subunit, or functional fragment thereof. In some cases, the therapeutic transgene is ATP7A, ATP7B, ATP8B1, ABCB4, ABCB11, or a variant, subunit, or functional fragment thereof. In some cases, the therapeutic transgene is CDKL5, CNTNAP2, ZEB2, or a variant or functional fragment thereof. In some cases, the transgene is a fibrinogen, prothrombin, a coagulation factor, or a blood clotting factor (e.g., Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12), or a variant or functional fragment thereof.

[0018] In some cases, any one of the expression vectors or cassettes as described herein is a viral particle, or is delivered using a viral particle, or is encapsidated or provided in a viral particle. In some cases, the viral particle is an adeno-associated virus, or an AAV. In some cases, the AAV is selected from the group consisting of: AAV1, AAV2, AAV3, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV-DJ, and scAAV. In other cases, the viral particle is a lentivirus. In some cases, the viral particle is an adenovirus.

[0019] In some instances, any one of the expression vectors or cassettes disclosed herein comprises a transgene that is a therapeutic transgene. In some cases, the therapeutic transgene is Factor VIII, Cas9, a DNA binding protein, hormone, growth or differentiation factor, insulin, growth hormone, VEGF, neurotrophic factor, fibroblast epithelial factor; cytokine, interleukin, lymphokine, tumor necrosis factor, antibody, immunoglobulin, interferon, chimeric T cell receptor; lipoprotein receptor, cystic fibrosis transmembrane regulator, a gene associated with mucopolysaccharidosis type I, II, III, or IV, beta globin or lipoprotein lipase, or a variant or functional fragment thereof. In some cases, the therapeutic transgene is ATP7A, ATP7B, ATP8B1, ABCB4, ABCB11, or a variant, subunit, or functional fragment thereof. In some cases, the transgene is any one of ATP7A, ATP7B, AT8B1 (or ATP8B1), MDR3 (or ABCB4), ABCBB (or ABCB11), CDKL5, CNTP2 (or CNTNAP2), ZEB2, Factor V, Factor VIII, Factor IX, or Factor X, or a variant, subunit, or functional fragment thereof. In some cases, the therapeutic transgene is CDKL5, CNTNAP2, ZEB2, or a variant, subunit, or functional fragment thereof. In some cases, the transgene is a fibrinogen, prothrombin, a coagulation factor, or a blood clotting factor (e.g., Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12), or a variant or functional fragment thereof.

[0020] In some instances, a method for delivering a transgene to a plurality of different tissues or cell types in an animal (e.g., a mammal, a human, a mouse, etc.) comprises administering to said animal any one of the expression vectors disclosed herein. In some cases, the administering comprises a systemic administration, e.g., via oral, intravenous, intramuscular, intraperitoneal, intrathecal, enteral, or parenteral administration.

[0021] In some instances, a method for production of proteins, antibodies, or other biologics, comprises contacting a cell with any one of the expression vectors disclosed herein. In some cases, the cell used for production of proteins is a CHO cell or a HEK293T cell. In some cases, an expression construct of any embodiment disclosed herein is used for a gene therapy treatment, e.g., expression in hepatocytes, kidney cells, and/or neurons (or in the central nervous system (CNS)). In other cases, an expression construct of any embodiment

disclosed herein is used to produce a protein ex vivo, e.g., in any mammalian or any human cell line, such as kidney cells, epithelial cells, liver cells, hepatocytes, neurons, fibroblasts, or CNS cells.

[0022] In some instances, a method for producing a transgenic animal or plant comprises administering to an animal or plant any one of the expression vectors or cassettes disclosed herein. In some cases, the regulatory element used in any one of the expression vectors or cassettes disclosed herein is relatively short, or 40-50bp, 50-60bp, 60-70bp, 70-80bp, 80-90bp, 90-100bp, 100-110bp, 110-120bp, or 120-130bp. In some cases, a RE in an expression cassette disclosed herein is 49bp, 56bp, 100bp, 117bp, 259bp, or 266bp. In some cases, the regulatory element used in any one of the expression vectors or cassettes disclosed herein is 45-50bp, 50-60bp, 45-60bp, 90-117bp, 95-110bp, 115-120bp, 250-260bp, or 260-270bp. In some cases, the regulatory element used in any one of the expression vectors or cassettes disclosed herein is about 100bp. In some cases, the regulatory element used in any one of the expression vectors or cassettes disclosed herein is less than or equal to 300bp, 250bp, 200bp, 150bp, 140bp, 130bp, 120bp, 110bp, 100bp, 70bp, or 50bp. In some cases, the regulatory element used in any one of the expression vectors or cassettes disclosed herein is 100bp or smaller. In some cases, two or more relatively short REs disclosed herein are combined and are operably linked to a transgene to drive high expression of the transgene. In some cases, two or more REs can be combined without any intervening nucleotides, or are combined using a linker of 1-50bp.

[0023] In some aspects, an expression cassette disclosed herein comprises a human-derived regulatory element operably linked to a therapeutic transgene, wherein the regulatory element comprises one or more of (i) SEQ ID NOs: 1-2, 13-17, and 22-41; (ii) a fragment or a combination thereof; or (iii) sequences having at least 80% sequence identity to any one of (i) and (ii). In some cases, the regulatory element is derived from human sequences of hg19. In some cases, the regulatory element is non-naturally occurring. In some cases, the regulatory element comprises an intronic sequence. In some cases, intronic sequence is located between a promoter and the therapeutic transgene. In some cases, the regulatory element comprises a promoter sequence. In some cases, the regulatory element is the only promoter in the cassette. In some cases, the regulatory element is no more than 100bp. In some cases, the regulatory element is no more than 60bp. In some cases, the regulatory element is no more than 50bp. In some cases, the expression cassette is part of a rAAV. In some cases, the rAAV is rAAV8. In some cases, the therapeutic transgene is ATP7B. In some cases, the therapeutic transgene is Factor VIII. In some aspects, a method of treating Wilson's disease comprises administering

an expression cassette disclosed herein and wherein the transgene is ATP7B. In some cases, a method of treating a haploinsufficiency or a genetic defect in ATP7B comprises administering an expression cassette disclosed herein and wherein the transgene is ATP7B. In some cases, a method of treating a blood clotting disorder comprises administering an expression cassette disclosed herein and wherein the transgene is Factor VIII. In some cases, a method of treating a haploinsufficiency or a genetic defect comprises administering an expression cassette disclosed herein and wherein the transgene is selected from: ATP7A; ATP7B; ATP8B1; ABCB4; ABCB11; CDKL5; CNTNAP2; ZEB2; Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12; and a variant or a functional fragment thereof. In some cases, a method of treating a haploinsufficiency or a genetic defect comprises administering an expression cassette disclosed herein and wherein the transgene is a transcriptional modulator that modulates expression of an endogenous gene. In some cases, a method of treating a haploinsufficiency or a genetic defect comprises administering the expression cassette disclosed herein and wherein the transgene is a transcriptional activator of any one of the following endogenous genes: ATP7A; ATP7B; ATP8B1; ABCB4; ABCB11; CDKL5; CNTNAP2; ZEB2; and Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12.

[0024] In some aspects, an AAV expression cassette comprises a human-derived regulatory element of no more than 120bp operably linked to a transgene of at least 3kb, wherein the regulatory element results in increased transgene expression by at least 2 fold as compared to expression of the transgene when operably linked to a CMV promoter. In some cases, the regulatory element is selected from: SEQ ID NO: 1-2, 13-17, and 22-41. In some cases, the regulatory element comprises (i) SEQ ID NO: 1-2, 13-17, and 22-41; (ii) a combination thereof; or (iii) or sequence having at least 80% sequence identity to any one of (i) and (ii). In some cases, the increased transgene expression is at least 50 fold. In some cases, the increased transgene expression is at least 100 fold. In some cases, the increased transgene expression occurs in at least 2 different cell types (e.g., hepatocytes and kidney cells). In some cases, the increased transgene expression occurs in at least 3 different cell types (e.g., hepatocytes, kidney cells, neurons, and/or epithelial cells). In some cases, the regulatory element comprises any one or more of SEQ ID NOs: 22-41, and wherein no other promoter sequences are present in the expression cassette. In some cases, the regulatory element is located upstream of the transgene. In some cases, the regulatory element comprises one or more of SEQ ID NO: 1 and SEQ ID NO: 2. In some cases, the regulatory element is located downstream of a promoter. In some cases, the transgene is selected from: ATP7A; ATP7B; ATP8B1; ABCB4; ABCB11; CDKL5; CNTNAP2; ZEB2; Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,

11, and 12; and a variant or a functional fragment thereof. In some cases, the transgene is ATP7A or ATP7B, or a variant or a functional fragment thereof. In some cases, the transgene is any one of Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12, or a variant or a functional fragment thereof. In some cases, the transgene is Factor 8, or a variant or a functional fragment thereof. In some cases, the transgene is a gene editing protein. In some cases, the gene editing protein is Cas (e.g., Cas9). In some cases, the transgene is a DNA binding protein. In some cases, the transgene is a transcriptional activator that increases expression of an endogenous gene. In some cases, the transgene is a transcriptional repressor that decreases expression of an endogenous gene. In some cases, the transcriptional activator increases expression of any one of the following endogenous genes: ATP7A; ATP7B; ATP8B1; ABCB4; ABCB11; CDKL5; CNTNAP2; ZEB2; and Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12. In some cases, the AAV is selected from the group consisting of: AAV1, AAV2, AAV3, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV-DJ, and scAAV.

[0025] In some aspects, a method of producing a recombinant protein comprises operably linking a sequence encoding the protein with one or more of (i) SEQ ID NO: 1-2, 13-17, and 22-41; (ii) a combination thereof; or (iii) sequences having at least 80% sequence identity to any one of (i) and (ii).

[0026] In some aspects, a method of treating a liver disease or condition comprises administering a gene therapy comprising a therapeutic transgene operably linked to one or more regulatory elements selected from (i) SEQ ID NO: 1-2, 13-17, and 22-41; (ii) a combination thereof; or (iii) sequences having at least 80% sequence identity to any one of (i) and (ii). In some cases, the liver disease or condition is Wilson's disease. In some cases, the liver disease or condition is a blood clotting disorder. In some cases, the transgene is ATP7A or ATP7B, or a variant or a functional fragment thereof. In some cases, the transgene is Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or a variant or a functional fragment thereof. In some cases, the transgene is Factor 8, or a variant or a functional fragment thereof. In some cases, the regulatory elements result in increased transgene expression in at least 2 cell types. In some cases, the regulatory elements result in increased transgene expression in at least 3 cell types. In some cases, the regulatory elements result in increased transgene expression in hepatocytes. In some cases, the regulatory elements result in increased transgene expression at a level that is at least 2 fold as compared to expression of the transgene when operably linked to a CMV promoter. In some cases, the gene therapy is AAV. In some cases, the AAV is AAV8.

[0027] In some aspects, an expression vector comprises a human-derived regulatory element having less than or equal to 100bp operably linked to a transgene, wherein the regulatory element increases global expression of the transgene by at least two fold as compared to a second expression vector without the regulatory element. In some cases, the second expression vector comprises the transgene operably linked to a CMV promoter.

[0028] In some aspects, an expression vector comprises a human-derived regulatory element having less than or equal to 100bp operably linked to a transgene, whereby expression of the transgene is higher than that of the same transgene expressed by a CMV promoter, super core promoter, TTR promoter, Proto 1 promoter, UCL-HLP promoter, or CMVe promoter. In some cases, the expression of the transgene is higher than that of the same transgene expressed by a UCL-HLP promoter. In some aspects, an expression vector comprises a human-derived regulatory element operably linked to a transgene, wherein a protein encoded by the transgene has (i) a concentration >1.0 IU/mL as measured by an ELISA assay configured to detect the transgene; and/or (ii) > 25% activity as measured by a Coatest assay. In some aspects, a vector disclosed herein comprises a human-derived regulatory element having a sequence less than or equal to 100bp in size operably linked to a transgene not found in context with the regulatory element in a cell or in vivo. In some cases, a vector disclosed herein comprises a regulatory element that is an intronic sequence. In some cases, the regulatory element is SEQ ID NO: 1 or SEQ ID NO: 2, or a sequence having at least 80% homology thereto. In some cases, use of a luciferase as the transgene results in global expression that is greater than $1x10^8$ photons/sec as measured in whole mice. In some cases, the activity or transgene expression corresponds to a dose of 16 µg of expression vector per mouse. In some cases, the activity or transgene expression corresponds to a dose of 12 µg of expression vector per mouse. In some cases, the endogenous version of the transgene is not linked to the regulatory element in vivo. In some cases, the global expression of the transgene is at a level greater than expression of the transgene using a vector with a regulatory element selected from the group consisting of: a CMV promoter, a CMVe promoter, a super core promoter, TTR promoter, Proto 1 promoter, and a UCL-HLP promoter. In some cases, expression is detectable in at least 2, 3, 4, 5, 6, or 7 different cell types in a mouse, in vivo, or in cell lines. In some cases, the different cell types are selected from the group consisting of: alveolar cells, cardiomyocytes, epithelial cells, hepatocytes, intestinal cells, myocytes, neurons, and renal cells. In some cases, the regulatory element is an enhancer. In some cases, a vector disclosed herein further comprises a promoter. In some cases, the promoter is a CMV promoter, CMV, promoter, super core promoter, TTR

promoter, Proto 1 promoter, UCL-HLP promoter, an AAT promoter, a KAR promoter, a EF1α promoter, EFS promoter, or CMVe enhancer/CMV promoter combination. In some cases, a vector disclosed herein further comprises one or more post-transcriptional modification sites. In some cases, the transgene is Cas9. In some cases, the transgene is saCas9.

[0029] In some aspects, an expression vector comprises a Factor VIII transgene operably linked to a regulatory sequence that is able to drive expression of the Factor VIII to a concentration >1.0 IU/mL as measured by an ELISA assay configured to detect Factor VIII in vivo. In some cases, a viral particle comprises an expression vector disclosed herein. In some cases, the viral particle is an AAV. In some cases, the AAV is selected from the group consisting of: AAV1, AAV2, AAV3, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV-DJ, and scAAV. In some cases, the viral particle is a lentivirus. In some cases, the virus particle is an adenovirus. In some cases, the transgene is a therapeutic transgene. In some cases, the therapeutic transgene is Factor VIII, Cas9, a DNA binding protein, hormone, growth or differentiation factor, insulin, growth hormone, VEGF, neurotrophic factor, fibroblast epithelial factor; cytokine, interleukin, lymphokine, tumor necrosis factor, antibody, immunoglobulin, interferon, chimeric T cell receptor; lipoprotein receptor, cystic fibrosis transmembrane regulator, a gene associated with mucopolysaccharidosis type I, II, III, or IV, beta globin, or lipoprotein lipase. In some cases, the therapeutic transgene is ATP7A, ATP7B, ATP8B1, ABCB4, ABCB11, or a variant or functional fragment thereof. In some cases, the therapeutic transgene is CDKL5, CNTNAP2, ZEB2, or a variant or functional fragment thereof. In some cases, a method for delivering a transgene to a plurality of different tissues or cell types in an animal comprises administering to said animal an expression vector disclosed herein. In some cases, a method for production of proteins comprises transfecting a cell with an expression vector disclosed herein. In some cases, the cell is a CHO cell or a HEK293T cell. In some cases, a method for producing a transgenic animal or plant comprises administering to an animal or plant an expression vector of any embodiment disclosed herein. In some cases, the regulatory element is 40-50bp. In some cases, the regulatory element is 50-60bp.

[0030] In some aspects, an expression vector comprises a human-derived regulatory element operably linked to a transgene, wherein a protein encoded by the transgene has (i) a concentration >0.1 IU/mL as measured by an ELISA assay configured to detect the transgene; and/or (ii) > 10% activity as measured by a Coatest assay. In other aspects, an expression vector comprises a human-derived regulatory element having <120bp operably

linked to a transgene, whereby expression of the transgene is higher than that of the same transgene expressed by a UCL-HLP promoter. In some cases, the regulatory element is a promoter. In some cases, the therapeutic transgene is a fusion protein comprising a DNA binding domain and a transcription regulatory domain.

INCORPORATION BY REFERENCE

[0031] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0033] **FIG. 1A** illustrates the normalized luciferase values of plasmids comprising different regulatory elements (e.g., SEQ ID NO: 1 and SEQ ID NO: 2), demonstrating the effect the REs had on expression of luciferase in HEK293T cells. For example, SEQ ID NO: 3 (comprising SEQ ID NO: 1 combined with a minimal CMV (minCMV) promoter) drove expression of luciferase at a level about 1.4 fold higher than the expression driven by the minCMV promoter alone, and about 60 fold higher than the expression driven by a SCP promoter.

[0034] **FIG. 1B** illustrates the size-normalized activity (calculated by dividing the normalized luciferase activity by the length of the regulatory element in base pairs) of each regulatory element (e.g., SEQ ID NO: 3 and SEQ ID NO: 4) as compared to SCP, minCMV, and CAG in HEK293T cells. SEQ ID NO: 4 (comprising SEQ ID NO: 2 linked to a minCMV promoter) resulted in size-normalized activity at a level about 3.5 fold higher than the minCMV promoter alone and about 140 fold higher than a SCP promoter.

[0035] **FIG. 1C** illustrates normalized luciferase expression of regulatory elements SEQ ID NOs: 17 and 22 combined, SEQ ID NOs: 16 and 23 combined, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33 as compared to negative controls and a positive control (SEQ ID NO: 4). All

the REs resulted in high levels of normalized luciferase expression that were comparable to the level of expression drove by SEQ ID NO: 4.

[0036] **FIG. 1D** illustrates normalized luciferase expression of regulatory elements SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 26, or SEQ ID NO: 27 as compared to a negative control, positive control (SEQ ID NO: 4), CMV+CMVe, and CMV alone in HEK293T cells. Each regulatory element drove higher luciferase expression than CMV alone and CMV+CMVe.

[0037] **FIG. 1E** illustrates normalized luciferase expression of regulatory elements SEQ ID NOs: 17 and 22 combined, SEQ ID NOs: 16 and 23 combined, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, or SEQ ID NO: 41 as compared to a negative control in HEK293T cells. Each regulatory element tested drove higher luciferase expression than the negative control.

[0038] **FIG. 2A** illustrates the expression of luciferase in mice dosed with expression cassettes comprising luciferase under the control of different regulatory elements (SCP, SerpE_TTR, Proto1, minCMV, UCL-HLP, CMVe, SEQ ID NO: 3, or SEQ ID NO: 4).

[0039] **FIG. 2B** illustrates the quantification of the average total luminescence (photons/sec) from luciferase expression observed in 3 mice injected with the expression cassettes of **FIG. 2A**.

[0040] **FIG. 3A** illustrates the effect of various regulatory elements (UCL-HLP, SEQ ID NO: 3, or SEQ ID NO: 4) on the expression of human factor 8 (FVIII) in mice as measured by an enzyme-linked immunosorbent assay. Expression cassettes comprising either SEQ ID NO: 3 or SEQ ID 4 resulted in elevated levels of human FVIII (IU/mL).

[0041] **FIG. 3B** illustrates the effect of various regulatory elements (UCL-HLP, SEQ ID NO: 3, or SEQ ID NO: 4) on expression of human factor 8 (FVIII) in mice as measured by the Coatest assay, which assayed the percent activity of human FVIII.

[0042] **FIG. 4A** illustrates gene editing performed in the dente gyrus of a mouse brain with a single AAV containing a Cas9 enzyme, a guide RNA, and one of several regulatory elements (minCMV, EFS, or SEQ ID NO: 4) to delete a termination sequence provided upstream of tdTomato (also referred to as red fluorescent protein (RFP)). RFP is represented by light gray color. An expression cassette comprising SEQ ID NO: 4 resulted in gene editing that led to higher expression of RFP as compared to the negative control, minCMV, and EFS.

[0043] **FIG. 4B** illustrates the quantification of the average number of RFP-expressing cells in **FIG. 4A**.

[0044] **FIG. 5A** illustrates an example of gene therapy used to treat a blood clotting deficiency in FVIII knockout mice in vivo using an rAAV expression cassette comprising a regulatory element of this disclosure. Blood loss (g) results of mice treated with PBS control or rAAV comprising a regulatory element of this disclosure at a dose of 3^{11} genome copies/kg (gc/kg) or at a dose of 3^{12} gc/kg were compared to untreated non-knockout mice. Treatment of mice with the rAAV gene therapy at the dose of 3^{12} gc/kg resulted in a reduction in blood loss as compared to the controls (e.g., PBS control mice).

[0045] **FIG. 5B** illustrates the bleeding time of the mice in minutes (min) till clotting.

[0046] **FIG. 6A** illustrate an exemplary expression cassette, vector, or plasmid of this disclosure, comprising AAV ITR-L and ITR-R and one or more regulatory elements, e.g., an enhancer, a promoter, stability elements, and polyA signal, operably linked to a transgene. Such rAAV vector can be used for gene therapy.

[0047] **FIG. 6B** illustrates a close-up view of an expression cassette wherein regulatory element SEQ ID NO: 1 is operably linked to a transgene, and wherein the regulatory element is located downstream of a promoter and upstream of the gene and a polyadenylation signal.

[0048] **FIG. 6C** illustrates an expression cassette wherein regulatory element SEQ ID NO: 2 is operably linked to a transgene, and wherein the regulatory element is located downstream of a promoter and upstream of the gene and a polyadenylation signal.

[0049] **FIG. 7** illustrates an exemplary expression cassette, vector, or plasmid of this disclosure, comprising AAV ITR-L and ITR-R and one or more relatively short human-derived regulatory elements of this disclosure (e.g., SEQ ID NOs: 13-17 and 22-41) operably linked to a large transgene, wherein the transgene is at least 3kb or 3-5kb in size. Such rAAV vector can be used for a gene therapy treatment.

[0050] **FIG. 8** illustrates the concentration of human FVIII (IU/mL) expressed in mice treated with various expression cassettes of this disclosure, e.g., expression cassettes comprising any one of regulatory elements SEQ ID NOs: 13-17, as compared to the untreated knockout mouse and SEQ ID NO: 9 (UCL-HLP).

[0051] **FIG. 9** illustrates the percent activity of human FVIII in mice treated with various expression cassettes of this disclosure, wherein SEQ ID NOs: 13-17 refer to a regulatory element.

[0052] **FIG. 10** illustrates blood loss experiments, measured in grams (g), with mice treated with various expression cassettes of this disclosure, wherein SEQ ID NOs: 13-17 refer to a regulatory element.

[0053] **FIG. 11** illustrates the bleeding time in minutes (min.) till clotting in mice treated with various expression cassettes of this disclosure, wherein SEQ ID NOs: 13-17 refer to a regulatory element.

[0054] **FIG. 12** illustrates the relative expression of ATP7B (Log_2) in mice treated with an expression cassette comprising a regulatory element having a sequence of SEQ ID NO: 13 at doses of 1E10 (or 10^{10}) genomic copies per mouse (gc/mouse), 1E11 (or 10^{11}) gc/mouse, or 1E12 (or 10^{12}) gc/mouse. The data showed a positive correlation between the dose of the expression cassette comprising SEQ ID NO: 13 and the expression of ATP7B in vivo.

[0055] **FIG. 13** illustrates the relative expression of EGFP (Log10) in mice treated with an expression cassette comprising a regulatory element having a sequence of SEQ ID NO: 4, 13, 14, 15, 16, 17, or 27 and administered at a dose of 5E11 gc/mouse. The data showed the REs resulted in elevated levels of EGFP expression, as measured by RNA transcripts, in the liver of the mice in vivo.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0056] The present disclosure contemplates compositions and methods for high expression of a transgene. Also described herein are compositions and methods for high expression of a transgene comprising one or more human or human-derived regulatory elements (REs) which, when operably linked to a transgene, can facilitate or result in high (or increased) expression of the transgene in one or more target cell types or tissues. In some embodiments, one or more regulatory elements disclosed herein drive high expression of a transgene in a cell or in vivo, in vitro, and/or ex vivo. In some embodiments, an expression cassette or vector comprising one or more regulatory elements described herein operably linked to a transgene can be adapted for use in any expression system or gene therapy to drive high expression of the transgene in a cell, a subject, or an animal model, or to result in a therapeutically effective level of transgene expression in a cell, a subject, or an animal model. In some embodiments, one or more REs of this disclosure is operably linked to a large transgene (e.g., a transgene whose sequence is more than 1 kb, 1.5kb, 2kb, 2.5kb, 3kb, 3.5kb, 4kb, 4.5kb, 5kb, 5.5kb, 6kb, 6.5kb, 7kb, or 7.5kb) to result in high expression of the transgene in a cell or in vivo. In some cases, such high expression of the transgene in a cell or in vivo is relative to expression of the transgene without said REs, wherein expression of the transgene with the REs is at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 15 fold, at least 20 fold, at least 25 fold, at least 50 fold, at least 100 fold, at least 150

fold, at least 200 fold, at least 250 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, at least 1000 fold, at least 1010 fold, at least 1020 fold, at least 1030 fold, at least 1040 fold, or at least 1050 fold as compared to transgene expression without the REs, or as compared to transgene expression by a negative control (e.g., buffer alone, vector alone, or a vector comprising a sequence known to have no expression activity).

[0057] In some cases, one or more REs result in high transgene expression in at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 different cell types. In some cases, one or more REs of this disclosure are operably linked to a transgene for a gene therapy treatment adapted for systemic administration. In some cases, one or more REs of this disclosure are operably linked to a transgene for a gene therapy treatment adapted for expression in the liver or hepatocytes. In some cases, the transgene is a gene that is expressed in the liver or in hepatocytes, or is a gene relevant in a liver disease or condition, e.g, ATP7B associated with Wilson's disease; ABCB4 associated with progressive familial intrahepatic cholestasis type 3; ALDOB associated with hereditary fructose intolerance; GBE1 associated with glycogen storage disease type IV; FAH associated with tyrosinemia type I; ASL associated with argininosuccinate lyase deficiency; SLC25A13 associated with citrin deficiency (CTLN2, NICCD); LIPA associated with cholesteryl ester storage disease; SERPINA1 associated with alpha-1 antitrypsin deficiency; CFTR associated with cystic fibrosis; HFE associated with hereditary hemochromatosis; or ALMS1 associated with Alström syndrome. In some cases, one or more regulatory elements disclosed herein are used for a gene therapy treatment to treat an inherited liver disease, e.g., a disorder of bile acid synthesis, a disorder of carbohydrate metabolism, a disorder of amino acid metabolism, a urea cycle disorder, or a disorder of lipid metabolism. In some cases, the transgene is a DNA binding protein, e.g., a transcriptional modulator, which modulates an endogenous gene. In some cases, a RE of any embodiment disclosed herein comprises a promoter sequence, an intronic sequence, a 5' UTR sequence, or any combination thereof. In some cases, a RE of any embodiment disclosed herein is a human-derived sequence (or a sequence that has at least 80%, 90%, 95% or 99% sequence identity to a sequence in a human reference genome). In some cases, a RE of any embodiment disclosed herein is less than or equal to 50bp, 60bp, 100bp, 120bp, 260bp, or 270bp in length. In some cases, a RE of any embodiment disclosed herein is less than or equal to 300bp, 250bp, 200bp, 150bp, 140bp, 130bp, 120bp, 110bp, 100bp, 70bp, or 50bp. In some cases, an expression cassette (e.g., a vector, an AAV, or a viral vector) comprises one or more RE sequences operably linked to a transgene of this

disclosure, wherein each RE sequence is any one of the sequences listed at **TABLE 1**, or a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity (e.g., local sequence identity) to a sequence of **TABLE 1**. In some cases, an expression construct (e.g., a vector, an AAV, or a viral vector) comprises one or more RE sequences operably linked to a transgene of this disclosure, wherein each RE sequence is no more than 50bp, 60bp, 70bp, 80bp, 90bp, 100bp, 110bp, 120bp, 130bp, 140bp, 150bp, 160bp, 170bp, 180bp, 190bp, 200bp, 210bp, 220bp, 230bp, 240bp, 250bp, 260bp, 270bp, 280bp, 290bp, 300bp, 310bp, 320bp, 330bp, 340bp, 350bp, 360bp, 370bp, 380bp, 390bp, or 400bp, and comprises a sequence according to any one or more of the sequences of **TABLE 1**. In some cases, an expression cassette (e.g., a vector, an AAV, or a viral vector) comprises one or more RE sequences operably linked to a transgene of this disclosure, wherein each RE sequence is no more than 50bp, 60bp, 70bp, 80bp, 90bp, 100bp, 110bp, 120bp, 130bp, 140bp, 150bp, 160bp, 170bp, 180bp, 190bp, 200bp, 210bp, 220bp, 230bp, 240bp, 250bp, 260bp, 270bp, 280bp, 290bp, 300bp, 310bp, 320bp, 330bp, 340bp, 350bp, 360bp, 370bp, 380bp, 390bp, or 400bp, and comprises a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% local sequence identity to any one of the sequences listed at **TABLE 1**. In some cases, local sequence identity is measured using BLAST. In some cases, a RE sequence of any embodiment disclosed herein is located upstream or downstream of a transgene. In some cases, two or more REs of any embodiment disclosed herein are operably linked to a transgene, wherein at least one or more of the REs are located upstream, downstream, or both upstream and downstream of the transgene.

[0058] In some cases, one or more REs of any embodiment of this disclosure are operably linked to a transgene to drive global expression of the transgene, wherein global expression refers to transgene expression in at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 different cell types. In some cases, one or more REs of any embodiment of this disclosure are operably linked to a transgene to drive global expression of the transgene when delivered systemically into a subject or animal. In some cases, systemic delivery or administration of an expression cassette disclosed herein is delivery via oral, intravenous, intramuscular, intraperitoneal, intrathecal, enteral, and/or parenteral administration. In various embodiments, a RE of any embodiment disclosed herein is human-derived (or a sequence that has at least 80%, 90%,

95% or 99% sequence identity to a sequence in a human reference genome). In some cases, a RE of any embodiment disclosed herein is non-naturally occurring.

[0059] Genetic engineering methods can replace a gene, modify a gene, or add a gene to achieve a desired result. Gene therapy is the use of genetic engineering to treat a disease or disorder. Genetic engineering may also be used to modify cells or organisms for the production of one or more proteins of interest. One challenge of genetic engineering is having regulatory elements that drive expression at a sufficiently high level to achieve a therapeutic effect, or at a sufficiently high level to achieve an industrial/production level. Another challenge of genetic engineering is designing an expression cassette small enough to fit within a desired vector, but containing a full coding sequence for a protein of interest and sufficient regulatory sequences to ensure high expression. The cloning capacity of vectors or viral expression vectors is a particular challenge for expression of large transgenes. For example, AAV vectors have a packaging capacity of ~4.8kb, lentiviruses have a capacity of ~8kb, adenoviruses have a capacity of ~7.5kb and alphaviruses have a capacity of ~7.5 kb. Some viruses have larger packaging capacities, for example herpesvirus with a capacity of >30kb and vaccinia with a capacity of ~25kb, however there may be advantages to using other viruses. For example, advantages of AAVs include low pathogenicity, very low frequency of integration into the host genome, and the ability to infect dividing and non-dividing cells.

[0060] One challenge in gene therapy is ensuring that the transgene is expressed at a sufficiently high level in at least one or more target cell types or tissues to result in a therapeutic effect in vivo. In some cases, increased transgene expression is desired in at least 2, 3, 4, 5, 6, 7, 8, 9, 10 or more different cell types or tissues, e.g., kidney cells, epithelial cells, liver cells, hepatocytes, neurons, fibroblasts, and/or CNS cells. In some cases, increased transgene expression is desired in at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 cell lines ex vivo for protein synthesis, e.g., a mammalian or a human cell line, such as kidney cells, epithelial cells, liver cells, hepatocytes, neurons, fibroblasts, immune cells, and/or CNS cells. Traditional methods for gene therapy have often relied on delivery methods and/or vehicles (e.g., varying the viruses used or capsid sequences of viruses). One technical challenge in the field is increasing level of gene expression, especially when the transgene is large, in one or more cell types or tissues to exert a therapeutic effect. Expression of large transgenes is associated with technical challenges because vectors, e.g., viral or AAV vectors, have limited cloning capacities. In order to express larger transgenes (e.g., a transgene whose sequence is more than 1kb, 1.5kb, 2kb, 2.5kb, 3kb, 3.5kb, 4kb, 4.5kb, 5kb, 5.5kb, 6kb, 6.5kb, 7kb, or

7.5kb), smaller regulatory elements must be used to accommodate the larger transgene in the vector. Thus, there is a need for novel short regulatory elements (e.g., REs that are less than or equal to 300bp, 250bp, 200bp, 150bp, 140bp, 130bp, 120bp, 110bp, 100bp, 70bp, or 50bp) that result in increased transgene expression in a cell or in vivo as compared to expression without the REs; a negative control (e.g., a sequence known to not drive any expression, an empty vector, or buffer alone control), or as compared to a promoter operably linked to the transgene (e.g., a CMV promoter, SCP, UCL-HLP, etc.).

[0061] As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising".

[0062] The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within one or more than one standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, up to 15%, up to 10%, up to 5%, or up to 1%) of a given value.

[0063] The terms "determining", "measuring", "evaluating", "assessing", "assaying", "analyzing", and their grammatical equivalents can be used interchangeably herein to refer to any form of measurement, and include determining if an element is present or not (for example, detection). These terms can include both quantitative and/or qualitative determinations. Assessing may be relative or absolute.

[0064] The term "expression" refers to the process by which a nucleic acid sequence or a polynucleotide is transcribed from a DNA template (such as into mRNA or other RNA transcript) and/or the process by which a transcribed mRNA is subsequently translated into peptides, polypeptides, or proteins. Transcripts and encoded polypeptides may be collectively referred to as "gene product." If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell. In some cases, global expression is desired and refers to expression of a gene (e.g., transgene) in at least 2, 3, 4, 5, 6, 7, 8, 9, 10 or more different cell types or tissues, or expression of a gene in a plurality of cell types. Cell types are distinguished by having a different cell marker, morphology, phenotype, genotype, function, and/or any other means for classifying cell types. In some cases, different cell types or tissues refer to cell types or tissues in vivo. In some cases,

different cell types or tissues refer to cell types or tissues ex vivo. In some cases, different cell types or tissues include, but are not limited to, different mammalian cell lines, human cell lines, kidney cells, epithelial cells, liver cells, hepatocytes, neurons, fibroblasts, and/or CNS cells.

[0065] As used herein, "operably linked", "operable linkage", "operatively linked", or grammatical equivalents thereof refer to juxtaposition of genetic elements, e.g., a promoter, an enhancer, a polyadenylation sequence, etc., wherein the elements are in a relationship permitting them to operate in the expected manner. For instance, a regulatory element, which can comprise promoter and/or enhancer sequences, is operatively linked to a coding region if the regulatory element helps initiate transcription of the coding sequence. There may be intervening residues between the regulatory element and coding region so long as this functional relationship is maintained.

[0066] A "vector" as used herein refers to a macromolecule or association of macromolecules that comprises or associates with a polynucleotide and which can be used to mediate delivery of the polynucleotide to a cell. Examples of vectors include plasmids, viral vectors, liposomes, and other gene delivery vehicles. The vector generally comprises genetic elements, e.g., regulatory elements, operatively linked to a gene to facilitate expression of the gene in a target. The combination of regulatory elements and a gene or genes to which they are operably linked for expression is referred to as an "expression cassette".

[0067] The term "AAV" is an abbreviation for adeno-associated virus, and may be used to refer to the virus itself or a derivative thereof. The term covers all serotypes, subtypes, and both naturally occurring and recombinant forms, except where required otherwise. The abbreviation "rAAV" refers to recombinant adeno-associated virus, also referred to as a recombinant AAV vector (or "rAAV vector"). The term "AAV" includes AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, rh10, and hybrids thereof, avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, non-primate AAV, and ovine AAV. The genomic sequences of various serotypes of AAV, as well as the sequences of the native terminal repeats (TRs), Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. A "rAAV vector" as used herein refers to an AAV vector comprising a polynucleotide sequence not of AAV origin (i.e., a polynucleotide heterologous to AAV), typically a sequence of interest for the genetic transformation of a cell. In general, the heterologous polynucleotide is flanked by at least one, and generally by two, AAV inverted terminal repeat sequences (ITRs). The term rAAV vector encompasses both rAAV vector

particles and rAAV vector plasmids. An rAAV vector may either be single-stranded (ssAAV) or self-complementary (scAAV). An "AAV virus" or "AAV viral particle" or "rAAV vector particle" refers to a viral particle composed of at least one AAV capsid protein and an encapsidated polynucleotide rAAV vector. If the particle comprises a heterologous polynucleotide (i.e., a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a mammalian cell), it is typically referred to as an "rAAV vector particle" or simply an "rAAV vector". Thus, production of rAAV particle necessarily includes production of rAAV vector, as such a vector is contained within an rAAV particle.

[0068] As used herein, the terms "treat", "treatment", "therapy" and the like refer to obtaining a desired pharmacologic and/or physiologic effect, including, but not limited to, alleviating, delaying or slowing the progression, reducing the effects or symptoms, preventing onset, inhibiting, ameliorating the onset of a diseases or disorder, obtaining a beneficial or desired result with respect to a disease, disorder, or medical condition, such as a therapeutic benefit and/or a prophylactic benefit. "Treatment," as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease or at risk of acquiring the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease. A therapeutic benefit includes eradication or amelioration of the underlying disorder being treated. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the underlying disorder such that an improvement is observed in the subject, notwithstanding that the subject may still be afflicted with the underlying disorder. In some cases, for prophylactic benefit, the compositions are administered to a subject at risk of developing a particular disease, or to a subject reporting one or more of the physiological symptoms of a disease, even though a diagnosis of this disease may not have been made. The methods of the present disclosure may be used with any mammal. In some cases, the treatment can result in a decrease or cessation of symptoms (e.g., a reduction in the frequency or duration of seizures). A prophylactic effect includes delaying or eliminating the appearance of a disease or condition, delaying or eliminating the onset of symptoms of a disease or condition, slowing, halting, or reversing the progression of a disease or condition, or any combination thereof.

[0069] The term "effective amount" or "therapeutically effective amount" refers to that amount of a composition described herein that is sufficient to affect the intended application, including but not limited to disease treatment, as defined below. The therapeutically effective

amount may vary depending upon the intended treatment application (in a cell or in vivo), or the subject and disease condition being treated, e.g., the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The term also applies to a dose that will induce a particular response in a target cell. The specific dose will vary depending on the particular composition chosen, the dosing regimen to be followed, whether it is administered in combination with other compounds, timing of administration, the tissue to which it is administered, and the physical delivery system in which it is carried.

[0070] A "fragment" of a nucleotide or peptide sequence is meant to refer to a sequence that is less than that believed to be the "full-length" sequence.

[0071] A "variant" of a molecule refers to allelic variations of such sequences, that is, a sequence substantially similar in structure and biological activity to either the entire molecule, or to a fragment thereof. As used herein, a variant of a gene refers to a sequence having a genetic alternation or a mutation as compared to the most common wild-type DNA sequence (e.g., cDNA, or the sequence referenced by its GenBank or sequence corresponding to a protein sequence referenced by its UniProt accession number). A gene variant encompasses mutants, such as a mutant that has an enhanced activity or function as compared to the wild-type sequence; any known subunit of a protein; and known isoforms of a gene that result from alternative splicing.

[0072] The term "functional fragment" is intended to include the "fragments", "variants", "analogues", or "chemical derivatives" of a molecule. A "functional fragment" of a DNA or protein sequence possesses at least a biologically active fragment of the sequence, which refers to a fragment that retains a biological activity (either functional or structural) that is substantially similar to a biological activity of the full-length DNA or protein sequence. A biological activity of a DNA sequence can be its ability to influence expression in a manner known to be attributed to the full-length sequence. For example, a functional fragment of a regulatory element will retain the ability to influence transcription as the full-length RE.

[0073] The terms "subject" and "individual" are used interchangeably herein to refer to a vertebrate, preferably a mammal, more preferably a human. "Subject" refers to an animal, such as a mammal, for example a human. The methods described herein can be useful in human therapeutics, veterinary applications, and/or preclinical studies in animal models of a disease or condition. In some cases, the subject is a mammal, and in some cases, the subject is human.

[0074] The term "in vivo" refers to an event that takes place in a subject's body.

[0075] The term "in vitro" refers to an event that takes places outside of a subject's body. For example, an in vitro assay encompasses any assay run outside of a subject. In vitro assays encompass cell-based assays in which cells alive or dead are employed. In vitro assays also encompass a cell-free assay in which no intact cells are employed.

[0076] Sequence comparisons, such as for the purpose of assessing identities, mutations, or where one or more positions of a test sequence fall relative to one or more specified positions of a reference sequence, may be performed by any suitable alignment algorithm, including but not limited to the Needleman-Wunsch algorithm (see, e.g., the EMBOSS Needle aligner available at www.ebi.ac.uk/Tools/psa/emboss_needle/, optionally with default settings), the BLAST algorithm (see, e.g., the BLAST alignment tool available at blast.ncbi.nlm.nih.gov/Blast.cgi, optionally with default settings), and the Smith-Waterman algorithm (see, e.g., the EMBOSS Water aligner available at www.ebi.ac.uk/Tools/psa/emboss_water/, optionally with default settings). Optimal alignment may be assessed using any suitable parameters of a chosen algorithm, including default parameters.

[0077] In general, "sequence identity" or "sequence homology", which can be used interchangeably, refer to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Typically, techniques for determining sequence identity include determining the nucleotide sequence of a polynucleotide and/or determining the amino acid sequence encoded thereby, and comparing these sequences to a second nucleotide or amino acid sequence. Two or more sequences (polynucleotide or amino acid) can be compared by determining their "percent identity", also referred to as "percent homology". The percent identity to a reference sequence (e.g., nucleic acid or amino acid sequences), which may be a sequence within a longer molecule (e.g., polynucleotide or polypeptide), may be calculated as the number of exact matches between two optimally aligned sequences divided by the length of the reference sequence and multiplied by 100. Percent identity may also be determined, for example, by comparing sequence information using the advanced BLAST computer program, including version 2.2.9, available from the National Institutes of Health. The BLAST program is based on the alignment method of Karlin and Altschul, Proc. Natl. Acad. Sci. USA 87:2264-2268 (1990) and as discussed in Altschul, et al., J. Mol. Biol. 215:403-410 (1990); Karlin and Altschul, Proc. Natl. Acad. Sci. USA 90:5873-5877 (1993); and Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997). Briefly, the BLAST program defines identity as the number of identical aligned symbols (i.e., nucleotides or amino acids), divided by the

total number of symbols in the shorter of the two sequences. The program may be used to determine percent identity over the entire length of the sequences being compared. Default parameters are provided to optimize searches with short query sequences, for example, with the blastp program. The program also allows use of an SEG filter to mask-off segments of the query sequences as determined by the SEG program of Wootton and Federhen, Computers and Chemistry 17: 149-163 (1993). Ranges of desired degrees of sequence identity are approximately 80% to 100% and integer values there between. Typically, the percent identities between a disclosed sequence and a claimed sequence are at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%. In general, an exact match indicates 100% identity over the length of the reference sequence. In some cases, reference to percent sequence identity refers to sequence identity as measured using BLAST (Basic Local Alignment Search Tool). In other cases, ClustalW can be used for multiple sequence alignment.

[0078] Unless otherwise indicated, all terms used herein have the same meaning as they would to one skilled in the art and the practice of the present invention will employ conventional techniques of molecular biology, microbiology, and recombinant DNA technology, which are within the knowledge of those of skill of the art.

Regulatory Elements

[0079] Regulatory elements can function at the DNA and/or the RNA level. Regulatory elements can function to modulate gene expression selectivity in a cell type of interest. Regulatory elements can function to modulate gene expression at the transcriptional phase, post-transcriptional phase, or at the translational phase of gene expression. Regulatory elements include, but are not limited to, promoter, enhancer, intronic, or other non-coding sequences. At the RNA level, regulation can occur at the level of translation (e.g., stability elements that stabilize mRNA for translation), RNA cleavage, RNA splicing, and/or transcriptional termination. In some cases, regulatory elements can recruit transcriptional factors to a coding region that increase gene expression selectivity in a cell type of interest. In some cases, regulatory elements can increase the rate at which RNA transcripts are produced, increase the stability of RNA produced, and/or increase the rate of protein synthesis from RNA transcripts.

[0080] Regulatory elements are nucleic acid sequences or genetic elements which are capable of influencing (e.g., increasing) expression of a gene (e.g., a reporter gene such as EGFP or luciferase; a transgene; or a therapeutic gene) in one or more cell types or tissues. In some cases, a regulatory element can be a transgene, an intron, a promoter, an enhancer,

UTR, an inverted terminal repeat (ITR) sequence, a long terminal repeat sequence (LTR), stability element, posttranslational response element, or a polyA sequence, or a combination thereof. In some cases, the regulatory element is a promoter, an enhancer, an intronic sequence, or a combination thereof. In some cases, the regulatory element is derived from a human sequence (e.g., hg19).

[0081] One method of isolating a regulatory element includes isolating nuclei from one or more cell types from an animal model, which can be achieved by using an affinity purification method that isolates one or more tissues or cell types (e.g., beads that bind to certain cell types), using high-throughput natural priming and DNA synthesis to generate a pool of sequences from open chromatin regions in the nuclei, sequencing the pool of sequences to identify putative sequences that drive gene expression in one or more tissues or cell types, and expression in a reporter system in one or more cell lines in vitro and/or in an animal model. In some cases, two or more regulatory elements are combined to form a larger RE. In some cases, combined regulatory elements exhibit enhanced gene expression in a plurality of cell types, e.g., in at least 2, 3, 4, 5, 6, or more cell types. In some instances, regulatory elements are truncated one or more bases at a time to determine the minimal amount of sequence that retains its ability to increase transgene expression. Smaller regulatory elements that retain transgene expression activity are helpful for making gene therapy comprising a large transgene, or where the cloning capacity of a vector or a plasmid is limited in view of the size of a transgene that one wishes to deliver using gene therapy. For example, one or more REs of this disclosure, when operably linked to a transgene, drive increased transgene expression, wherein the transgene is at least 1 kb, 1.5kb, 2kb, 2.5kb, 3kb, 3.5kb, 4kb, 4.5kb, 5kb, 5.5kb, 6kb, 6.5kb, 7kb, or 7.5kb. In some cases, such increase in transgene expression is at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 15 fold, at least 20 fold, at least 25 fold, at least 50 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, or at least 1000 fold as compared to a negative control, a constitutive promoter, a CMV promoter, super core promoter, TTR promoter, Proto 1 promoter, UCL-HLP promoter, or CMVe promoter.

[0082] In some cases, a regulatory element of this disclosure results in high or increased expression of an operably linked transgene, wherein the high or increased expression is determined as compared to a control, e.g., a constitutive promoter, a CMV promoter, CAG, super core promoter (SCP), TTR promoter, Proto 1 promoter, UCL-HLP promoter, minCMV,

EFS, or CMVe promoter. Other controls that can be used to determine high or increased transgene expression by a regulatory element disclosed herein include buffer alone or vector alone. In some cases, a positive control refers to a RE with known expression activity, such as SEQ ID NO: 4, which can be used for comparison. In some cases, a regulatory element drives comparable or higher transgene expression as comparable to a positive control (e.g., SEQ ID NO: 4 or a known promoter operably linked to the transgene).

[0083] In some cases, the 5'-most 17 nucleotides of SEQ ID NOs: 13-17 can be removed to form shorter REs that retain the expression activity. For examples, SEQ ID NOs: 13-17 without the 5'-most 17 nucleotides are SEQ ID NOs: 30, 26, 28, 23, and 22, respectively.

[0084] In other cases, two or more relatively short REs can be combined to form a larger RE having high transgene expression activity and/or size-normalized gene expression activity. For examples, SEQ ID NO: 17 can be combined with SEQ ID NO: 22; or SEQ ID NO: 16 can be combined with SEQ ID NO: 22.

[0085] The present disclosure contemplates relatively short, human-derived regulatory elements that increase expression of an operably linked transgene. In some cases, a short human-derived RE is any one of the sequences at **TABLE 1**, or SEQ ID NOs: 1-2, 13-17, and 22-41.

[0086] TABLE 1: Examples of Regulatory Elements (REs)

SEQ ID NO:	Sequence (5'-to-3')	Length
1	GTAAGGTAAGAATTGAATTTCTCAGTTGAAGGATGCTTACACTCTT GTCCATCTAG	56bp
2	GTGTGTATGCTCAGGGGCTGGGAAAGGAGGGGAGGGAGCTCCGGC TCAG	49bp
3	GTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTG ACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAG TTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAAC AACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGG GAGGTCTATATAAGCAGAGCTGGTACCGTAAGGTAAGAATTGAAT TTCTCAGTTGAAGGATGCTTACACTCTTGTCCATCTAG	266bp
4	GTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTG ACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAG TTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAAC AACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGG GAGGTCTATATAAGCAGAGCTGGTACCGTGTGTATGCTCAGGGGC TGGGAAAGGAGGGGAGGGAGCTCCGGCTCAG	259bp
13	GTGATGACGTGTCCCATAAGGCCCCTCGGTCTAAGGCTTCCCTATT TCCTGGTTCGCCGGCGGCCATTTTGGGTGGAAGCGATAGCTGAGTG GCGGCGGCTGCTGATTGTGTTCTAG	117bp
14	GTGATGACGTGTCCCATACTTCCGGGTCAGGTGGGCCGGCTGTCTT GACCTTCTTTGCGGCTCGGCCATTTTGTCCCAGTCAGTCCGGAGGC TGCGGCTGCAGAAGTACCGCCTGCG	117bp
15	GTGATGACGTGTCCCATATTTTCATCTCGCGAGACTTGTGAGCGGC	117bp

SEQ ID NO:	Sequence (5'-to-3')	Length
	CATCTTGGTCCTGCCCTGACAGATTCTCCTATCGGGGTCACAGGGA CGCTAAGATTGCTACCTGGACTTTC	
16	GTGATGACGTGTCCCATGGCCTCATTGGATGAGAGGTCCCACCTCA CGGCCCGAGGCGGGGCTTCTTTGCGCTTAAAAGCCGAGCCGGGCC AATGTTCAAATGCGCAGCTCTTAGTC	117bp
17	GTGATGACGTGTCCCATCCCCCCTCCACCCCCTAGCCCGCGGAGCA CGCTGGGATTTGGCGCCCCCCTCCTCGGTGCAACCTATATAAGGCT CACAGTCTGCGCTCCTGGTACACGC	117bp
22	CCCCCCTCCACCCCCTAGCCCGCGGAGCACGCTGGGATTTGGCGCC CCCCTCCTCGGTGCAACCTATATAAGGCTCACAGTCTGCGCTCCTG GTACACGC	100bp
23	GGCCTCATTGGATGAGAGGTCCCACCTCACGGCCCGAGGCGGGGC TTCTTTGCGCTTAAAAGCCGAGCCGGGCCAATGTTCAAATGCGCAG CTCTTAGTC	100bp
24	GGGTGGGGCCCGCGCGTATAAAGGGGGCGCAGGCGGGCTGGGCG TTCCACAGGCCAAGTGCGCTGTGCTCGAGGGGTGCCGGCCAGGCC TGAGCGAGCGA	100bp
25	GGTGCGATATTCGGATTGGCTGGAGTCGGCCATCACGCTCCAGCTA CGCCACTTCCTTTTCGTGGCACTATAAAGGGTGCTGCACGGCGCTT GCATCTCT	100bp
26	ACTTCCGGGTCAGGTGGGCCGGCTGTCTTGACCTTCTTTGCGGCTC GGCCATTTTGTCCCAGTCAGTCCGGAGGCTGCGGCTGCAGAAGTA CCGCCTGCG	100bp
27	GCTGAGCGCGCGCGATGGGGCGGGAGGTTTGGGGTCAAGGAGCA AACTCTGCACAAGATGGCGGCGGTAGCGGCAGTGGCGGCGCGTAG GAGGCGGTGAG	100bp
28	ATTTTCATCTCGCGAGACTTGTGAGCGGCCATCTTGGTCCTGCCCT GACAGATTCTCCTATCGGGGTCACAGGGACGCTAAGATTGCTACCT GGACTTTC	100bp
29	TGGGACCCCCGGAAGGCGGAAGTTCTAGGGCGGAAGTGGCCGAG AGGAGAGGAGAATGGCGGCGGAAGGCTGGATTTGGCGTTGGGGCT GGGGCCGGCGG	100bp
30	AAGGCCCCTCGGTCTAAGGCTTCCCTATTTCCTGGTTCGCCGGCGG CCATTTTGGGTGGAAGCGATAGCTGAGTGGCGGCGGCTGCTGATT GTGTTCTAG	100bp
31	AGTGACCCGGAAGTAGAAGTGGCCCTTGCAGGCAAGAGTGCTGGA GGGCGGCAGCGGCGACCGGAGCGGTAGGAGCAGCAATTTATCCGT GTGCAGCCCC	100bp
32	GGGAGGGGCGCGCTGGGGAGCTTCGGCGCATGCGCGCTGAGGCCT GCCTGACCGACCTTCAGCAGGGCTGTGGCTACCATGTTCTCTCGCG CGGGTGTCG	100bp
33	ACTGCGCACGCGCGCGGTCGCACCGATTCACGCCCCCTTCCGGCG CCTAGAGCACCGCTGCCGCCATGTTGAGGGGGGGACCGCGACCAG CTGGGCCCCT	100bp
34	CCCTCGAGGGGCGGAGCAAAAAGTGAGGCAGCAACGCCTCCTTAT CCTCGCTCCCGCTTTCAGTTCTCAATAAGGTCCGATGTTCGTGTAT AAATGCTCG	100bp
35	CTTGGTGACCAAATTTGAAAAAAAAAAAAAACCGCGCCAACTCAT GTTGTTTTCAATCAGGTCCGCCAAGTTTGTATTTAAGGAACTGTTT CAGTTCATA	100bp
36	GGCTGAGCTATCCTATTGGCTATCGGGACAAAATTTGCTTGAGCCA ATCAAAGTGCTCCGTGGACAATCGCCGTTCTGTCTATAAAAAGGTG	100bp

SEQ ID NO:	Sequence (5'-to-3')	Length
	AAGCAGCG	
37	GGAAGTGCCAGACCGGAGGTGCGTCATTCACCGGCGACGCCGATACGGTTCCTCCACCGAGGCCCATGCGAAGCTTTCCACTATGGCTTCCAGCACTGTC	100bp
38	CCCTCGAGGGGCGGAGCAAAAAGTGAGGCAGCAACGCCTCCTTATCCTCGCTCCCGCTTTCAGTTCTCAATAAGGTCCGATGTTCGTGTATAAATGCTCG	100bp
39	CTTGGTGACCAAATTTGAAAAAAAAAAAAAACCGCGCCAACTCATGTTGTTTTCAATCAGGTCCGCCAAGTTTGTATTTAAGGAACTGTTTCAGTTCATA	100bp
40	GGCTGAGCTATCCTATTGGCTATCGGGACAAAATTTGCTTGAGCCAATCAAAGTGCTCCGTGGACAATCGCCGTTCTGTCTATAAAAAGGTGAAGCAGCG	100bp
41	GGAAGTGCCAGACCGGAGGTGCGTCATTCACCGGCGACGCCGATACGGTTCCTCCACCGAGGCCCATGCGAAGCTTTCCACTATGGCTTCCAGCACTGTC	100bp

[0087] In some cases, a relatively short human-derived RE comprises a sequence of **TABLE 1** or SEQ ID NOs: 1-2, 13-17, and 22-41; or a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity (e.g., local sequence identity) to a sequence of **TABLE 1** or SEQ ID NOs: 1-2, 13-17, and 22-41. In some cases, a short human-derived RE comprises a non-naturally occurring sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to a human sequence of hg19. In some cases, a relatively short human-derived RE comprises a non-naturally occurring sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to a human sequence located at one of the following genomic or chromosomal locations listed in **TABLE 2**.

[0088] TABLE 2: Examples of Genomic Locations for Deriving REs

SEQ ID NO:	Genomic location	Fold increase over negative control (normalized)
22	PEG10-cs;PEG10-he_chr7:94285387-94285887-3	154
23	SAT1-he_chrX:23801204-23801704-1	207
24	CDKN1C-he;CDKN1C-cs_chr11:2906751-2907251-3	532
25	BTG1-cs;BTG1-he_chr12:92539423-92539923-3	501
26	ATP5A1-he_chr18:43678011-43678511-3	1049

SEQ ID NO:	Genomic location	Fold increase over negative control (normalized)
27	GANAB-he_chr11:62413828-62414328-3	54
28	ATP5F1-he_chr1:111991856-111992356-3	675
29	LMAN2-cs_chr5:176778403-176778903-3	
30	RAB1A-he_chr2:65356988-65357488-3	1049
31	NEDD8-he_chr14:24701287-24701787-3	253
32	ATP5C1-he_chr10:7829892-7830392-3	
33	SSR4-he_chrX:153059879-153060379-1	
34	HIST1H4B-he_chr6:26027230-26027730(-)-2	
35	HIST1H4C-he_chr6:26103854-26104354(+)-3	313
36	HIST2H2AA3-he_chr1:149814228-149814728(-)-4	
37	TOMM6-he_chr6:41755153-41755653(+)-3	
38	HIST1H4B-he_chr6:26027230-26027730(-)-2	
39	HIST1H4C-he_chr6:26103854-26104354(+)-3	
40	HIST2H2AA3-he_chr1:149814228-149814728(-)-4	
41	TOMM6-he_chr6:41755153-41755653(+)-3	

[0089] In some cases, a relatively short human-derived RE is derived from a promoter at a genomic location listed in **TABLE 2** and exhibits at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 15 fold, at least 20 fold, at least 25 fold, at least 50 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, or at least 1000 fold transgene expression as compared to a negative control (e.g., vector alone control, buffer control, or a sequence known to have no expression activity), or as compared to a constitutive promoter, a CMV promoter, super core promoter, TTR promoter, Proto 1 promoter, UCL-HLP promoter, or CMVe promoter.

[0090] In some cases, a relatively short RE of this disclosure comprises an intronic sequence (e.g., SEQ ID NO: 1 or SEQ ID NO: 2), when operably linked to a transgene in an expression cassette, results in normalized gene (e.g., luciferase, ATP7B, or FVIII) expression of at least 50 fold, 100 fold, 500 fold, 1000 fold, or 5000 fold, or more than 1000 fold as compared to transgene expression under the control of SCP or a negative control (e.g., vector alone, buffer alone, or a sequence without any expression activity). In some cases, a relatively short RE of this disclosure (e.g., SEQ ID NO: 1 or SEQ ID NO: 2), when operably linked to a transgene in an expression cassette, results in size-normalized activity that is at least 1.5 fold, 2.5 fold, 5 fold, 10 fold, 15 fold, or 20 or more fold as compared to the size-normalized activity of SCP or CAG. In some cases, a relatively short RE of this disclosure (e.g., SEQ ID NO: 1 or SEQ ID NO: 2), when operably linked to a transgene (e.g., luciferase) in an

expression cassette, results in total luciferase luminescence (in photons/sec) that is at least 1.5 fold, 2 fold, 2.5 fold, 3 fold, 4 fold, 5 fold, 10 or more fold as compared to luciferase under the control of SCP, SerpE_TTR, Proto1, minCMV, UCL-HLP, or CMVe; or as compared to a buffer control. In some cases, a relatively short RE of this disclosure (e.g., SEQ ID NO: 1 or SEQ ID NO: 2), when operably linked to human FVIII in an expression cassette, results in an increase in human FVIII concentration in vivo, wherein the increase in concentration is at least 1 IU/mL, 1.2 IU/mL, 1.5 IU/mL, or more than 1 IU/mL of FVIII as compared to buffer control or UCL-HLP operably linked to FVIII.

[0091] In some cases, a relatively short RE of this disclosure is derived from a human promoter sequence (e.g., SEQ ID NOs: 13-17 and 22-41), when operably linked to a transgene (e.g., luciferase, ATP7B, or FVIII) in an expression cassette, results in normalized transgene expression of at least 50 fold, 100 fold, 200 fold, 300 fold, 400 fold, 500 fold, 600 fold, 700 fold, 800 fold, 900 fold, or 1000 or more fold as compared to a negative control (e.g., vector alone, buffer alone, or a sequence known to have no expression activity). In some instances, the RE results in normalized luciferase expression of at least 5 fold, 10 fold, or 100 or more fold as compared to luciferase expression under the control of CMV or CMV+CMVe. In some embodiments, two or more REs can be combined to form a larger RE. In some cases, the RE is located upstream of the transgene.

[0092] In some cases, a relatively short human-derived RE disclosed herein comprises no more than 40bp, 45bp, 49bp, 50bp, 56bp, 60bp, 70bp, 80bp, 90bp, 100bp, 110bp, 117bp, 120bp, 130bp, 140bp, 150bp, 160bp, 170bp, 180bp, 190bp, 200bp, 210bp, 220bp, 230bp, 240bp, 250bp, 259bp, 260bp, 265bp, 270bp, 280bp, 290bp, 300bp, 310bp, 320bp, 330bp, 340bp, 350bp, 360bp, 370bp, 380bp, 390bp, or 400bp, and comprises a sequence according to any one of **TABLE 1** or SEQ ID NOs: 1-2, 13-17, and 22-41. In some cases, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more of the sequences listed in **TABLE 1**, or SEQ ID NOs: 1-2, 13-17, and 22-41, can be combined or used in tandem in an expression cassette or vector.

[0093] In various aspects, relatively short, human-derived regulatory elements are especially useful when there is a size constraint on a vector (such as in AAV gene therapy) or a need for a very high level of expression (such as in ex vivo protein production). The regulatory elements disclosed herein may be constitutive or inducible. Thus, the present disclosure provides the benefits of both high expression as well as short regulatory elements. In some cases, a RE disclosed herein exhibits higher expression level per base pair or per nucleotide as compared to a control regulatory element, such as a constitutive promoter, a

CMV promoter, super core promoter, TTR promoter, Proto 1 promoter, UCL-HLP promoter, or CMVe promoter. In some cases, SEQ ID NO: 4 is used as a control for comparing levels of gene expression.

[0094] In some cases, one or more REs of this disclosure are adapted for increasing expression of a large transgene (e.g., in a gene therapy or an expression cassette) because the REs enhance transgene expression without taking up significant cloning capacity. In some cases, a RE of this disclosure is no more than 49bp, 50bp, 56bp, 60bp, 70bp, 80bp, 90bp, 100bp, 110bp, 117bp, 120bp, 130bp, 140bp, 150bp, 160bp, 170bp, 180bp, 190bp, 200bp, 210bp, 220bp, 230bp, 240bp, 250bp, 259bp, 260bp, 265bp, 270bp, 280bp, 290bp, 300bp, 310bp, 320bp, 330bp, 340bp, 350bp, 360bp, 370bp, 380bp, 390bp, or 400bp, and comprises a sequence according to any one of **TABLE 1** or SEQ ID NOs: 1-2, 13-17, and 22-41, or a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto. In some cases, a relatively short RE of this disclosure increases transgene expression by at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 15 fold, at least 20 fold, at least 25 fold, at least 50 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, or at least 1000 fold as compared to transgene expression without the RE, or as compared to a negative control (e.g., vector alone control, buffer alone, or a sequence known to have no expression activity). In some cases, a control is a constitutive promoter operably linked to the same transgene (e.g., luciferase). Examples of other promoters that can be used as a control for comparison to transgene expression of a RE operably to the transgene include, but are not limited to, a CMV promoter, a super core promoter, a TTR promoter, a Proto 1 promoter, a UCL-HLP promoter, an AAT promoter, a KAR promoter, a EF1α promoter, EFS promoter, or CMVe enhancer/CMV promoter combination.

[0095] A regulatory element herein can be a promoter, and when included in an expression cassette will drive transcription of a downstream sequence, which may be closely associated or in direct contact with the downstream sequence (e.g., a transgene). A promoter may drive high, medium, or low expression of a linked transgene. In some cases, a promoter may be specific for one or more cell types, or may express at a similar level in many or a plurality of cell types (e.g., in at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 cell types).

[0096] In some cases, a RE disclosed herein comprises a human-derived sequence. In some cases, a RE of this disclosure is non-naturally occurring. The term "human-derived" as used herein refers to a sequence that has at least 80%, 90%, 95% or 99% sequence identity to a sequence in a human reference genome (or a human genome build, such as hg19). A homologous sequence may be a sequence which has a region with at least 80% sequence identity (e.g., as measured by BLAST) as compared to a region of the human genome. For example, a sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% homologous to a human sequence is deemed a human derived sequence.

[0097] In some cases, a regulatory element contains a human-derived sequence and additional sequences such that overall the regulatory element has low sequence identity to a sequence in the human genome, while a part of the regulatory element has 100% sequence identity (or local sequence identity) to a sequence in the human genome. In some instances, percent sequence identity refers to the homology within a region covering at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of any of the sequences listed in **TABLE 1** or SEQ ID NOs: 1-2, 13-17, and 22-41.

[0098] In some cases, a human-derived regulatory element is a sequence that is 100% identical to a human sequence. In some instances, the sequence of a regulatory element is 100% human derived, wherein the RE differs from the human sequence by at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95 nucleotides or base pairs.

[0099] In other instances, at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% of the regulatory element sequence is human derived. For example, a regulatory element can have 50% of its sequence be human derived, and the remaining 50% be non-human derived (e.g., mouse derived or fully synthetic). For further example, a regulatory element that is regarded as 50% human derived and comprises 300bp may have an overall 45% sequence identity to a sequence in the human genome, while base pairs 1-150 of the RE may have 90% identity (e.g., local sequence identity) to a similarly sized region of the human genome.

[00100] In some cases, a RE of this disclosure comprises a promoter sequence, an intronic sequence, an enhancer sequence, a 5' UTR sequence, or a combination thereof. In some cases, a RE of this disclosure is non-naturally occurring. In some cases, a RE of this

disclosure is human-derived (or a sequence that has at least 80%, 90%, 95% or 99% sequence identity to a sequence in a human reference genome).

[00101] In some cases, a relatively short regulatory element disclosed herein may be derived from a genomic promoter sequence (e.g., genomic locations in **TABLE 2**). In some cases, a regulatory element disclosed herein may be derived from both genomic promoter sequence and 3' untranslated region (3' UTR). In some cases, a regulatory element disclosed herein may be derived from intergenic sequence. In some cases, a regulatory element disclosed herein may be derived from genomic sequence downstream of a gene, or from 5' UTR sequence, or a mixture of 5' UTR and downstream sequence.

[00102] A regulatory element herein can be an enhancer, and its presence in an expression vector or cassette along with a promoter increases expression of an operably linked transgene as compared to expression of the same transgene by the promoter without the enhancer. An enhancer may increase expression of a linked transgene through either a transcriptional mechanism, posttranscriptional mechanism, or both.

[00103] In some cases, a regulatory element herein is an intronic sequence (e.g., SEQ ID NOs: 1-2), or comprises an intron, and its presence in an expression vector or cassette along with a promoter increases expression of an operably linked transgene as compared to expression of the same transgene by the promoter without the intronic sequence. An intronic sequence or regulatory element can increase expression of a linked transgene through either a transcriptional mechanism, posttranscriptional mechanism, or both.

[00104] In some cases, a regulatory element herein is a promoter sequence (e.g., SEQ ID NOs: 13-17 and 22-41), or comprises a promoter sequence, and it can be operably linked to a transgene in an expression cassette without any other promoter sequences and/or enhancer sequences to express the transgene.

[00105] In various embodiments, one or more of REs of **TABLE 1** can drive increased expression of an operably linked transgene without the need for a promoter in an expression cassette. In some embodiments, where a large transgene (e.g., a transgene whose sequence is more than 1 kb, 1.5kb, 2kb, 2.5kb, 3kb, 3.5kb, 4kb, 4.5kb, 5kb, 5.5kb, 6kb, 6.5kb, 7kb, or 7.5kb) is too large to be expressed in a conventional expression cassette, such transgene can be operably linked to one or more REs of **TABLE 1** without the need for a conventional promoter and be expressed at higher levels as compared to transgene expression in an expression cassette without the REs, or as compared to a control (e.g., an empty vector, a constitutive promoter, a CMV promoter, super core promoter, TTR promoter, Proto 1 promoter, UCL-HLP promoter, or CMVe promoter).

[00106] One of the benefits of the regulatory elements disclosed herein is their relatively short length. In some instances, a regulatory element of the invention is less than or equal to 40bp, 50bp, 60bp, 70bp, 80bp, 90bp, 100bp, 200bp, 300bp, or 400bp in length. In some instances, a regulatory element is less than or equal to100bp in length, <90bp, <80bp, <70bp, <60bp, or <50bp, or between 48-57bp, or between 40-60bp, or 50-100bp in length. In some cases, the regulatory element described herein is 40-50bp, 45-55bp, 50-60bp, or 55-65bp. In some cases, the regulatory element is 45-60bp. In some cases, the regulatory element described herein is 49bp or 56bp. For example, regulatory elements SEQ ID NO: 1 is 56bp in length, and regulatory element SEQ ID NO: 2 is 49bp in length. In some cases, the regulatory element may be between 100bp and 150bp, between 110bp and 140bp, between 110bp and 130bp, or between 115bp and 125bp. For example, regulatory elements SEQ ID NOs. 13-17 are 117bp in length. In some cases, regulatory elements are or are about 100bp. For example, SEQ ID NOs: 22-41 are each 100bp in length.

[00107] A regulatory element of the disclosure preferably has a human-derived sequence. A regulatory element may be derived from a genomic sequence upstream of a transcription initiation site, a 5' UTR sequence, an exonic sequence, an intronic sequence, or a 3' UTR sequence. In some embodiments, a human-derived regulatory element is an intronic human-derived sequence. In some cases, a human-derived regulatory element is a promoter sequence derived from a human sequence. In some embodiments, a human-derived regulatory element is an intronic sequence of SEQ ID NO: 1, SEQ ID NO: 2, or a combination thereof, or a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto (e.g., as measured by BLAST). In some embodiments, a human-derived regulatory element is a promoter sequence of SEQ ID NO: 13, 14, 15, 16, 17, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, or a combination thereof, or a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto (e.g., as measured by BLAST).

[00108] In some cases, the regulatory elements comprise part or all of a 5' untranslated region (5' UTR). 5' UTR regulatory elements can influence expression of a gene in several different ways. 5' UTR regulatory elements can contain binding sites for RNA binding proteins. Further, secondary structures formed by regulatory elements in the 5' UTR can affect the binding of RNA binding proteins required for translation. In some examples, the

regulatory element can have a high degree of secondary structure. In some cases, the regulatory element can have little or no secondary structure. The regulatory element can also contain an internal ribosome entry site (IRES), allowing for 5' cap independent translation. The regulatory element can contain an upstream translation initiation codon (uAUG). In some embodiments, the regulatory element does not contain an upstream translation initiation codon. In some embodiments, the regulatory element does not contain any codon within one base of an AUG codon, or contains fewer codons similar to an AUG codon than expected by chance. In some cases, the regulatory element can contain an upstream open reading frame, which occurs when an upstream AUG (or sufficiently similar sequence) is present, followed by an in frame stop codon. In some examples, the regulatory element does not comprise an uORF. In some cases, the regulatory elements contain microRNA binding sites, or binding sites for RNA binding proteins.

[00109] In some instances, a regulatory element of the disclosure comprises a sequence that is homologous to a human-derived sequence. A sequence is "homologous to a human-derived sequence" if it has at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity to a human sequence. Preferably, a sequence that is homologous to a human-derived regulatory sequence is at least 90% identical to a human sequence. In some embodiments, a regulatory element herein is one that has at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity to SEQ ID NOs: 1-2, 13-17, and 22-41. When a regulatory element is homologous to any of SEQ ID NOs: 1-2, such regulatory element still results in higher expression of an operably linked transgene (when a promoter is also present in the expression vector or cassette), as compared to a similar vector without the regulatory element. Such higher expression of a transgene can be observed, e.g., in expression in HEK293T or CHO cells. In some cases, such higher expression of a transgene can be observed in a plurality of cell types, e.g., two or more mammalian cell lines, human cell lines, kidney cells, epithelial cells, liver cells, hepatocytes, neurons, fibroblasts, and/or CNS cells.

[00110] In some cases, when a regulatory element is homologous to any of SEQ ID NOs: 13-17 and 22-41, such regulatory element results in higher expression of an operably linked transgene (even when no other promoter is present in the expression vector or cassette), as compared to a similar vector without the regulatory element. Such higher expression of a transgene can be observed, e.g., in expression in HEK293T or CHO cells. In some cases, such higher expression of a transgene can be observed in a plurality of cell types, e.g., two or more mammalian cell lines, human cell lines, kidney cells, epithelial cells, liver cells, hepatocytes, neurons, fibroblasts, and/or CNS cells.

[00111] A regulatory element of the disclosure can also be a functional fragment of any of the above, e.g., a fragment of SEQ ID NOs: 1-2, 13-17, and 22-41, or any combination thereof. Such a functional fragment also increases expression of a transgene in an expression cassette or vector when compared to a similar expression cassette or vector without the regulatory element. When the functional fragment is an enhancer, intronic sequence, a promoter sequence, or a combination thereof, higher expression is observed when the fragment is operably linked to a transgene, as compared to a similar vector or cassette without the functional fragment. A fragment is preferably less than or equal to 25bp, 30bp, 40bp, 50bp, 60bp, 70bp, 80bp, 90bp, 100bp, or 110bp in length. In some cases, a RE of this disclosure derived from a human promoter sequence can increase expression of an operably linked transgene without the need for a second promoter in an expression cassette or vector. In some cases, a promoter in a conventional expression cassette or vector can be replaced with one or more SEQ ID NOs: 13-17 and 22-41, a combination thereof, or a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity (e.g., as measured by BLAST) to SEQ ID NOs: 13-17 and 22-41, or a combination thereof.

[00112] In some embodiments, a regulatory element is any one of: (i) SEQ ID NO: 1, 2, 3, 4, 13, 14, 15, 16, 17, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, (ii) a nucleic acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NO: 1, 2, 3, 4, 13, 14, 15, 16, 17, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, (iii) a functional fragment of any sequence of (i) or (ii), or (iv) a combination of any sequence of (i), (ii) and (iii). In some embodiments, two or more copies of a regulatory element are used to enhance transgene expression, e.g., two or more copies of one or more of SEQ ID NOs: 1-2, 13-17, and 22-41. In some embodiments, a combination of SEQ ID NOs: 1 and 2, or a combination of functional fragments thereof, is used as a regulatory element to increase transgene expression. In other embodiments, one or more of SEQ ID NO: 1 and/or 2 are coupled or operably linked to another regulatory element, such as a promoter or enhancer, to increase transgene expression. In other embodiments, one or more of SEQ ID NO: 1 and/or 2 are coupled or operably linked to one or more of SEQ ID NOs: 1-2, 13-17, and 22-41 to increase transgene expression. In some embodiments, a regulatory element that is an intronic sequence can increase transgene expression when the regulatory element is coupled or operably linked to any promoter. In

some cases, a regulatory element that is derived from a human promoter sequence can increase transgene expression when the regulatory element is coupled or operably linked to a transgene without any other promoter sequences. In some cases, a regulatory element comprising a human-derived promoter sequence and an intronic sequence can increase transgene expression when the combined regulatory element is coupled or operably linked to a transgene without any other promoter sequences.

[00113] In some embodiments, one can enhance any gene therapy or known gene therapy by adding one or more regulatory elements (e.g., SEQ ID NOs: 1-2, 13-17, and 22-41) as disclosed herein to improve or increase transgene expression from the gene therapy.

[00114] In some instances, a regulatory element contains a human-derived sequence and a non-human-derived sequence such that overall the regulatory element has low sequence identity to the human genome. However a part of the regulatory element has 100% sequence identity to the human genome. In other instances, at least 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% of the regulatory element sequence is human-derived or at least 10, 20, 30, 40, or 50 contiguous nucleotides are human-derived. For example, a regulatory element can have 50% of its sequence be human-derived, and the remaining 50% be non-human-derived (e.g., mouse derived, virus derived or fully synthetic).

[00115] An increase in expression can occur at the transcriptional or posttranscriptional level. For example, at the transcriptional level, a regulatory element can increase expression by recruiting transcription factors, and/or RNA polymerase, increasing initiation of transcription or recruiting DNA and/or histone modifications that increase the level of transcription. Such increases of expression can be detected by measuring an increase in the amount of RNA transcripts that are representative of the transgene. At the posttranscriptional level, a regulatory element can increase expression by increasing the amount of protein that is translated into protein. This can be achieved through various mechanisms, for example, by increasing the stability of the mRNA or increasing recruitment and assembly of proteins required for translation. Such increase of expression can be detected by measuring the amount of protein expressed that is representative of the transgene. The amount of protein produced can be measured directly, for example by an enzyme linked immunosorbent assay (ELISA), or indirectly, for example, by a functional assay. A protein commonly measured in a functional assay is luciferase. However, other proteins such as Factor VIII can also be measured by functional assays. Preferably, the regulatory elements herein increase expression of a gene, a transgene, or a therapeutic transgene of an expression vector or cassette by at least 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, or 10 fold, or more than 5 fold, or

more than 10 fold, as compared to expression of the transgene using the same expression vector minus the regulatory element. In some cases, the regulatory elements herein increase expression of a gene, a transgene, or a therapeutic transgene of an expression vector or cassette by at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 15 fold, at least 20 fold, at least 25 fold, at least 50 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, or at least 1000 fold as compared to expression of the transgene using the same expression vector minus the regulatory element. Such expression vectors or cassettes can include a promoter, separate from the regulatory element. Examples of promoters include: a minCMV promoter, a super core promoter (SCP), a TTR promoter, a Proto 1 promoter, a UCL-HLP promoter, an AAT promoter, a KAR promoter, an EF1α promoter, and an EFS promoter. In some cases, an expression vector or cassette comprising one or more of SEQ ID NOs: 13-17 and 22-41 operably linked to a transgene does not need a separate promoter for transgene expression.

[00116] In some instances, expression of a transgene operably linked to any of the regulatory elements herein together with a minCMV promoter is higher than expression of the transgene linked to a minCMV promoter without the regulatory element described herein. In some cases, expression of the transgene when linked to a minCMV promoter and a regulatory element as described herein is higher than expression of the transgene when linked, without the regulatory element, to any one of the following promoters: a super core promoter, a TTR promoter, a Proto 1 promoter, a UCL-HLP promoter, an AAT promoter, a KAR promoter, an EF1α promoter, or an EFS promoter.

[00117] In some instances, expression of a transgene operably linked to any of the regulatory elements herein (e.g., SEQ ID NOs: 1-2, 13-17, and 22-41) is higher than the expression of the transgene when linked to a UCL-HLP promoter.

[00118] In some instances, the regulatory elements described herein drive high expression relative to their length. For example, a regulatory element of this disclosure that is half the length of a CMV promoter drives expression that is more than half the level of the CMV promoter. In some cases, a regulatory element is defined by the activity (or transgene expression) per base pair, or referred to as size-normalized activity. For example, a regulatory element which drives the same level of expression as a CMV promoter, but which is half the length of the CMV promoter has twice the activity per base pair. As shown in **FIG. 1A**, SEQ ID NO: 3 (SEQ ID NO: 1 combined with a minCMV promoter) results in a normalized

luciferase value of 5552, which yields a size-normalized activity value of ~21 as shown in **FIG. 1B** (SEQ ID NO: 3 is 266bp). SEQ ID NO: 4 (SEQ ID NO: 2 combined with a minCMV promoter) results in a size normalized value of 53. The minCMV promoter alone yields a size normalized value of 19, and the SCP promoter and CAG promoter yield size normalized values of 1 and 10, respectively, as shown in **FIG. 1B**.

[00119] Expression, high gene expression, in particular, can be measured using any technique known in the art. In some instances, the relative and higher expression of a transgene when controlled by a regulatory element of the disclosure is measured using RNA quantification/sequencing techniques, such as quantitative PCR, northern blotting, or next generation sequencing.

[00120] In some instances, high expression can be measured by the concentration of the protein produced/expressed from the transgene/gene of interest. The concentration of the protein can be measured by any method known in the art. Non-limiting examples of methods for measuring protein expression include, but not limited to, ELISA, radioimmunoassay, western blotting, or high performance liquid chromatography. See, for examples, Noble JE, *Quantification of protein concentration using UV absorbance and Coomassie dyes*, Methods Enzymol. 2014;536:17-26; Kurien BT, Scofield RH. *A brief review of other notable protein detection methods on acrylamide gels*, Methods Mol Biol. 2012;869:617-20; and Daniel M. Bollag, Michael D. Rozycki and Stuart J. Edelstein, *Protein Methods*, 2 ed., Wiley Publishers (1996). Protein expression can be measured in vitro or in vivo.

[00121] In various embodiments, transgene expression can be determined by measuring the amount of mRNA or RNA transcripts produced from the transgene using a PCR method. In some cases, transgene gene expression can be measured by using a protein or an immunoassay that interacts with the gene product. In some cases, Northern blot analysis and/or in situ hybridization can also be used to analyze transgene expression in vivo. The level of the protein expressed (e.g., fluorescence from a fluorescent reporter transgene) from the transgene can also be monitored to determine an increase in transgene expression in terms of transcription as well as an increase in protein synthesis (i.e., translation) in a cell or in vivo. Protein levels can also be assayed using various methods, including, but not limited to, Western blot analysis, immunohistochemistry, immunofluorescence histochemistry, and/or ELISA assays.

[00122] In some examples, a regulatory element of this disclosure results in expression of an operably linked transgene at a level of at least 0.5, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.5, or 3 IU/ml in a relevant cell type as measured by ELISA. Examples of relevant cell

types include, but are not limited to, hepatocytes, liver cells, muscle cells, lung cells, epithelial cells, and kidney cells, e.g., 293T and CHO cells. In some embodiments, a regulatory element's ability to increase transgene expression can be assessed in a mouse wherein the total amount of transgene expression in the whole mouse and/or the total number of cell types or tissue types having transgene expression are measured. In some cases, a regulatory element of the disclosure can result in expression of an operably linked transgene at an overall level of at least 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.5, or 3.0 IU/ml in the blood of a mouse or other organism, as measured by ELISA.

[00123] When assessing the activity of an expression cassette or vector, the activity or expression can be represented as an activity or expression level per unit dose, or normalized to a dose of expression cassette or vector administered or delivered into a cell or mouse. In some embodiments, expression or activity of a transgene is normalized to an amount of plasmid or DNA (e.g., µg/kg per mouse), or viral particles (e.g., normalized to an amount of genome copies/kg per mouse) used to allow comparison across different expression vectors or cassettes with or without a regulatory element. For example, when assessing a regulatory element's activity in a mouse, expression or activity assayed can be normalized to a dose of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or greater than 10 or 20 µg of expression vector, cassette, or plasmid per mouse. In some embodiments, the expression level or activity can be normalized to 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, or 10^{15} gc/kg of viral particles containing an expression vector or cassette as disclosed herein per mouse.

[00124] In other examples, expression of the linked transgene is measured by a functional assay for the activity of the expressed protein. Examples of functional assays include a luciferase assay for quantifying luciferase activity and the Coatest assay for quantifying Factor VIII activity. In the Coatest assay, expression of active Factor VIII, a co-factor, facilitates a reaction that releases a chromophoric group, pNA. The color associated with the chromophoric group released is read photometrically at 405 nm, and the intensity of the color is proportional to the amount of Factor VIII activity. Factor VIII activity can be measured as a percentage of the activity level shown by blood plasma containing 1.0 IU/mL of Factor VIII. A regulatory element of this disclosure, e.g., SEQ ID NO: 1 or 2, operably linked to minCMV and Factor VIII can result in Factor VIII activity level of more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 250% or more than 250% of the activity level as compared to a similar expression vector without the regulatory element, such as UCL-HLP without the

regulatory element, when the protein is at a concentration of 1.0 IU/mL. In some cases, a regulatory element of this disclosure, e.g., SEQ ID NO: 13, 14, 15, 16, 17, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, operably linked to Factor VIII can result in Factor VIII activity level of more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 250% or more than 250% of the activity level as compared to a similar expression vector with a UCL-HLP promoter, e.g., when the protein is at a concentration of 1.0 IU/mL.

[00125] The regulatory elements disclosed herein can drive or increase expression of a transgene in one or more different cell types. When a regulatory element is capable of increasing expression of a transgene in multiple cell types, such expression is deemed global expression. Global expression does not require expression of a transgene in every cell of an organism. Global regulatory elements can express a transgene at similar levels in different expressing cell types, or at a range of expression levels in different cell types. In some cases, global regulatory elements can drive or increase expression of a transgene in at least two, three, four, five, ten, fifteen, or twenty different cell types. For example, a regulatory element with global expression can drive or increase expression of a transgene in at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 different cell lines. Examples of cell lines include 293 cells, CHO cells, cancer cell lines, and immortalized cell lines. In some instances, a regulatory element drives or increases expression of a transgene in one or more cell types in an animal in vivo.

[00126] In some embodiments, a regulatory element herein drives expression of a gene in liver cells. In some embodiments, a regulatory element drives expression of a gene in any one or more of the following cell types: alveolar cells, cardiomyocytes, epithelial cells, hepatocytes, kidney cells, intestinal cells, myocytes, neurons, ovarian cells, and renal cells. In some embodiments, a regulatory element drives expression of a gene in any one or more of the following cell lines: Chinese hamster ovary cells (CHO), mouse myeloma cells (NSO), baby hamster kidney cells (BHK), B16 melanoma cells, HEK293T cells, HeLa cells, HT-1080 cells, and PER.C5 cells. In some instances, the regulatory elements drive expression of a gene in a cell line ex vivo or in vitro.

[00127] The regulatory elements of this disclosure can be combined together or combined with other regulatory elements. Such other regulatory elements include, for examples, a constitutive promoter, an inducible promoter, a repressor, an enhancer or a posttranscriptional stability element. In one embodiment, a vector comprises regulatory element of SEQ ID NO: 3, comprising SEQ ID NO: 1 and a minCMV promoter, upstream of a linked transgene. Examples of promoters contemplated herein include: minCMV promoter, super core

promoter, TTR promoter, Proto 1 promoter, UCL-HLP promoter, CMVe enhancer/CMV promoter combination, AAT promoter, KAR promoter, EF1α promoter, EFS promoter, or CBA promoter.

[00128] The combined regulatory elements of this disclosure can still be short. The combined regulatory elements can have a total length of about 50 to 500, 100 to 400, 50 to 200, 100 to 200, or 150 to 300 base pairs in length. The combined regulatory elements can have a combined length of less than about 500, 450, 400, 350, 300, 250, 200, 150 or 100 base pairs in length. In some embodiments, the regulatory element is 40-50bp, 45-50, 49, 50-60, 56, 50-55, or 45-60bp.

[00129] The combined regulatory elements can come from different species. In preferred examples, at least one part of a combined regulatory element is human-derived. Non-human-derived elements can be derived from mammalian, viral, or synthetic sequences.

Expression Cassettes

[00130] The terms "expression cassette" and "nucleic acid cassette" are used interchangeably to refer to a polynucleotide molecule or a nucleic acid sequence. In some cases, an expression cassette comprises one or more regulatory elements disclosed herein operably linked to a transgene. In some cases, an expression cassette comprises one or more regulatory elements. In some cases, the expression cassette further comprises a promoter. In some cases, an expression cassette comprises one or more sequences of SEQ ID NOs: 1-2, 13-17, and 22-41 and/or any combination thereof. In some cases, an expression cassette comprises one or more of SEQ ID NOs: 1-2, 13-17, and 22-41, (ii) a nucleic acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 1-2, 13-17, and 22-41, (iii) a functional fragment of any sequence of (i) or (ii), or (iv) a combination of any sequence of (i), (ii) and/or (iii). In some cases, sequence identity is measured by BLAST. In some cases, a regulatory element is located upstream of a transgene in an expression cassette. In some cases, a regulatory element is located downstream of a transgene in an expression cassette.

[00131] In some aspects, one or more regulatory elements described herein (e.g., SEQ ID NOs: 1-2, 13-17, and 22-41) are operably linked to a transgene (e.g., a reporter gene or a therapeutic transgene) in an expression cassette. In some cases, a gene therapy comprises an expression cassette comprising a transgene operably linked to one or more, two or more, three or more, four or more, or five or more regulatory elements of the present disclosure to result in increased expression of a transgene, e.g., a reporter gene, eGFP, RFP, Factor VIII,

Cas9, DNA binding protein, hormone, growth or differentiation factor, insulin, growth hormone, VEGF, neurotrophic factor, fibroblast epithelial factor; cytokine, interleukin, lymphokine, tumor necrosis factor, antibody, immunoglobulin, interferon, chimeric T cell receptor; lipoprotein receptor, cystic fibrosis transmembrane regulator, a gene associated with mucopolysaccharidosis type I, II, III, or IV, beta globin or lipoprotein lipase, or a variant or fragment thereof. In some cases, the transgene is ATP7A, ATP7B, ATP8B1, ABCB4, ABCB11, or a variant or functional fragment thereof, or sequences having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto. In some cases, the transgene is CDKL5, CNTNAP2, ZEB2, or a variant or functional fragment thereof, or sequences having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto. In some cases, the transgene is a fibrinogen, prothrombin, a coagulation factor, or a blood clotting factor (e.g., Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12), or a variant or functional fragment thereof.

[00132] In some cases, an expression cassette is adapted for delivery via gene therapy. In some cases, an expression cassette is a linear or a circular construct. In some cases, an expression cassette is part of a plasmid, vector, a viral vector, or rAAV. In some cases, an expression cassette comprising one or more REs of this disclosure operably linked to a transgene is adapted for expression in a plurality of cells, e.g., in any mammalian or human cell line, kidney cells, epithelial cells, liver cells, hepatocytes, neurons, fibroblasts, and/or CNS cells. In some cases, an expression cassette comprising one or more REs of this disclosure (e.g., SEQ ID NOs: 1-2, 13-17, and 22-41) operably linked to a transgene is adapted for expression in 2 or more, 3 or more, 4 or more, or 5 or more mammalian or human cell lines or cell types. In some cases, transgene expression occurs in 2 or more, 3 or more, 4 or more, or 5 or more of the following cell types: kidney cells, epithelial cells, hepatocytes, neurons, fibroblasts, cardiomyocytes, CNS cells, muscle cells, blood cells, and/or stem cells.

[00133] In some cases, an expression cassette comprising one or more REs of this disclosure (e.g., SEQ ID NOs: 1-2, 13-17, and 22-41) operably linked to a transgene is adapted for transgene expression in the liver or in hepatocytes. In some cases, an expression cassette comprising one or more REs of this disclosure operably linked to a transgene is adapted for systemic delivery of a transgene, e.g., via oral, intravenous, intramuscular, intraperitoneal, intrathecal, enteral, or parenteral administration. In some cases, systemic delivery of a transgene involves increased transgene expression in the liver or in hepatocytes. In some cases, an expression cassette comprising one or more REs of this disclosure operably linked to a transgene results in expression of the transgene in hepatocytes at a level that is at least

1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 15 fold, at least 20 fold, at least 25 fold, at least 50 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, or at least 1000 fold as compared to an expression cassette without the REs, or as compared to a control (e.g., a vector alone control, buffer control, or a sequence that does not have any expression activity). In some cases, the expression driven by a relatively short RE is compared to that of a constitutive promoter, a CMV promoter, super core promoter, TTR promoter, Proto 1 promoter, UCL-HLP promoter, or CMVe promoter operably linked to the same transgene.

[00134] In some cases, an expression cassette comprising a regulatory element of this disclosure results in high or increased expression of an operably linked transgene, wherein the high or increased expression is determined as compared to a control, e.g., a constitutive promoter, a CMV promoter, super core promoter (SCP), TTR promoter, Proto 1 promoter, UCL-HLP promoter, or CMVe promoter. Other controls that can be used for comparison to determine high or increased transgene expression include buffer alone, minCMV, EFS, vector alone, or a sequence known to have no expression activity. In some cases, CAG or SEQ ID NO: 4 is used as a positive control for comparing expression level and/or size-normalized activity.

[00135] Expression of a transgene or gene of interest can occur via an expression vector or expression cassette. Such expression vector or cassette can include any one or more of the following additional elements promoters, enhancers, or post-transcriptional regulatory elements. The regulatory elements of this disclosure can be positioned upstream or downstream of the transgene, and can be transcribed or not transcribed. In some examples, the regulatory elements of this disclosure are positioned downstream of a promoter and upstream of the transgene. In some examples, the regulatory elements are positioned as promoters of the transgene. In some cases, one or more REs of this disclosure are operably linked to a transgene without any other promoter in an expression vector or cassette.

[00136] Expression vectors or cassettes can be circular or linear nucleic acid molecules. In some cases, a vector or cassette is delivered to cells (e.g., a plurality of different cells or cell types, including target cells or cell types and/or non-target cells or cell types). A vector or cassette can be an integrating or non-integrating vector, referring to the ability of the vector or cassette to integrate the expression vector or cassette, in part or whole, and/or the transgene into a genome of a cell. Either an integrating vector or a non-integrating vector can be used to deliver a transgene operably linked to a regulatory element. Examples of vectors or

cassettes include, but are not limited to, (a) non-viral vectors such as nucleic acid vectors including linear oligonucleotides and circular plasmids; artificial chromosomes such as human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), and bacterial artificial chromosomes (BACs or PACs)); episomal vectors; transposons (e.g., PiggyBac); and (b) viral vectors such as retroviral vectors, lentiviral vectors, adenoviral vectors, and adeno-associated viral vectors (AAVs). Viruses have several advantages for delivery of nucleic acids, including high infectivity. In some embodiments, a virus is used to deliver a nucleic acid molecule or expression cassette comprising one or more regulatory elements, as described herein, operably linked to a transgene.

[00137] The vectors described herein can be delivered to a cell of interest either directly or via a delivery system. Examples of delivery systems include, but are not limited to, viral vectors (e.g., retroviral, adenoviral, adeno-associated (AAV), helper-dependent adenoviral systems, hybrid adenoviral systems, herpes simplex, pox virus, lentivirus, and Epstein–Barr virus), physical systems (naked DNA, DNA bombardment, electroporation, hydrodynamic, ultrasound, and magnetofection), and chemical systems (cationic lipids, different cationic polymers, and lipid polymers).

[00138] Any known technique can be used to deliver the regulatory element(s) and an operably linked transgene, or compositions comprising regulatory elements and a transgene, to the cells of interest to confer or induce in vitro, in vivo, or ex vivo expression of the transgene in a cell-type, tissue or organism of interest.

[00139] Preferred characteristics of viral gene therapy vectors or gene delivery vectors include the ability to be reproducibly and stably propagated and purified to high titers; to mediate targeted delivery (e.g., to deliver the transgene specifically to the tissue or organ of interest without widespread vector dissemination elsewhere); and to mediate gene delivery and transgene expression without inducing harmful side effects.

[00140] Several types of viruses, for example, the non-pathogenic parvovirus, adeno-associated virus, have been engineered for the purposes of gene delivery. Virus-based vectors for various gene-therapy applications can harness the viral infection pathway but avoid the subsequent expression of viral genes that can lead to replication and toxicity. Such virus-based vectors can be obtained by deleting all, or some, of the coding regions from the viral genome, but leaving intact those sequences (e.g., terminal repeat sequences) that are necessary for functions such as packaging the expression vector into the virus capsid or the integration of vector nucleic acid or heterologous gene or DNA into the host genome. An expression vector or cassette comprising a transgene, for example, can be cloned into a viral

backbone such as a modified or engineered viral backbone lacking viral genes, and used in conjunction with additional vectors (e.g., packaging vectors), which can, for example, when co-transfected, produce recombinant viral vector particles.

[00141] Several serotypes of AAV, a non-pathogenic parvovirus, have been engineered for the purposes of gene delivery, some of which are known to have tropism for certain tissues or cell types. Viruses used for various gene-therapy applications can be engineered to be replication-deficient or to have low toxicity and low pathogenicity in a subject or a host. Such virus-based vectors can be obtained by deleting all, or some, of the coding regions from the viral genome, and leaving intact those sequences (e.g., inverted terminal repeat sequences) that are necessary for functions such as packaging the vector genome into the virus capsid or the integration of vector nucleic acid (e.g., heterologous DNA sequence) into the host genome. An expression vector or cassette comprising a transgene, for example, can be cloned into a viral backbone such as a modified or engineered viral backbone lacking viral genes, and used in conjunction with additional vectors (e.g., packaging vectors), which can, for example, when co-transfected, produce recombinant viral vector particles.

[00142] In some embodiments, an AAV vector or an AAV viral particle, or virion, that is used to deliver one or more regulatory elements and a transgene into a cell, cell type, or tissue, in vivo, ex vivo, or in vitro, is preferably replication-deficient. In some embodiments, an AAV virus is engineered or genetically modified so that it can replicate and generate virions only in the presence of helper factors.

[00143] In some embodiments, the expression vector or cassette is designed for delivery by an AAV, or a recombinant AAV (rAAV). In some embodiments, an expression vector or cassette is delivered using a lentivirus or a lentiviral vector. In some embodiments, larger transgenes, i.e., genes that exceed the cloning capacity of an AAV, are preferably delivered using a lentivirus or a lentiviral vector.

[00144] The expression vector or cassette can be designed for delivery by an AAV. The AAV can be any serotype, for examples, AAV1, AAV2, AAV3, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV-DJ, or a chimeric, hybrid, or variant AAV. The AAV can also be a self-complementary AAV (scAAV). An expression vector or cassette designed for delivery by an AAV can comprise a 5' ITR, one or more regulatory elements, an optional minimal promoter, a transgene, optionally one or more introns, an optional polyA signal, and a 3' ITR. In some instances an expression vector or cassette also contains one or more post-transcriptional RNA regulatory elements.

[00145] Exemplary expression vectors or cassettes containing SEQ ID NO: 1 or SEQ ID NO: 2 are shown in **FIG. 6B** and **FIG. 6C**, respectively. An exemplary expression vector which could contain any one or more of SEQ ID NOs: 13-17 and 22-41 in the promoter position is shown in **FIG. 7**. Such expression vectors or cassettes can be adapted for transgene expression using any one of the delivery systems or gene therapy described herein, including, but not limited to, an AAV, lentivirus, adenovirus, or retrovirus.

[00146] The expression vector or cassette can be designed for delivery by an optimized therapeutic retroviral vector. The retroviral vector can be a lentivirus comprising a left (5') LTR; sequences which aid packaging and/or nuclear import of the virus, at least one nucleic acid cell-type selective regulatory element, optionally a lentiviral reverse response element (RRE); optionally a promoter or active portion thereof; a therapeutic transgene operably linked to the various regulatory elements; optionally an insulator; and a right (3') retroviral LTR.

[00147] In some embodiments, the expression vector or cassette comprises one or more regulatory elements. In some examples, the vector or cassette contains two or more regulatory elements combined. In some examples, the vector or cassette contain two or more regulatory elements which are not combined, for example, a promoter upstream of the transgene and an enhancer located downstream of the transgene. In some embodiments, an intron sequence is located downstream of a promoter.

[00148] In some cases, the expression vector or cassette contains a high driver regulatory element, a global regulatory element, or a short regulatory element. In some cases, the high driver regulatory element drives global expression and/or is short. In some cases, the regulatory element is a short global regulatory element. In some embodiments, the expression vector or cassette containing a regulatory element is used in a viral vector, or is packaged in a virus, which can be used to infect an animal model in vivo or to transfect cells ex vivo or in vitro to assess the activity of the regulatory element in the animal model as a whole or in certain cell types, or a target cell type.

[00149] In some embodiments, expression cassettes containing regulatory elements of this disclosure also comprise one or more transgenes. The transgenes can be protein-coding genes. In some cases, the expression cassette contains a therapeutic transgene. The therapeutic transgene can replace an absent or defective gene, or compensate for deficient expression of a protein. The therapeutic transgene can be involved in a cell signaling pathway. In some cases, the expression vector or cassette contains a transgene for commercial production. In some embodiments, the transgene for commercial production can

produce a therapeutic protein, or improve a function of a host cell, or restore or rescue a phenotype of a host cell.

[00150] The regulatory elements disclosed herein can be located at any position within an expression vector or cassette. For example, the regulatory elements can be positioned upstream of an enhancer, downstream of an enhancer but upstream of a promoter, within the 5' UTR of a transgene, within an intron within the transgene, in the 3' UTR of the transgene, or downstream of the transgene. In some cases, one or more regulatory elements are positioned upstream or downstream of the operably linked transgene. For example, SEQ ID NO: 3 and 4 contain the sequences of SEQ ID NO: 1 and 2, respectively, downstream of a minCMV promoter. Regulatory elements can have different effects on transgene expression from different locations within an expression cassette or vector. Each regulatory element can have a preferred location within the expression vector or cassette which results in optimal expression from that regulatory element.

[00151] Utilizing shorter regulatory elements with high activity can allow for delivery of a larger transgene within the same packaging capacity and without sacrificing transgene expression or high expression. In some cases, use of a shorter regulatory element allows for delivery of two or more transgenes in a single vector, or delivery of a large transgene at relatively higher levels of expression as compared to a similar vector or cassette without the regulatory element. The two or more transgenes can comprise two or more different protein-coding genes or a protein-coding gene and a non-coding element. In one example, a vector comprises a Cas9 coding sequence and one or more guide RNAs, as described in Example 4. In some cases, the regulatory elements are about 20 to 100, 30 to 100, 40 to 80, or 50 to 60 base pairs in length. In some cases, the regulatory elements are less than 150, 120, 110, 100, 90, 80, 70, or 60 base pairs in length. The combined regulatory elements of this disclosure can be short. The combined regulatory elements can have a total length of about 50 to 500, 100 to 400, 50 to 200, 100 to 200, or 150 to 300 base pairs in length. The combined regulatory elements can have a combined length of less than about 500, 450, 400, 350, 300, 250, 200, 150, or 100 base pairs in length.

[00152] In some cases, an expression cassette or vector comprises one or more REs of this disclosure operably linked to a large transgene whose sequence is more than 1kb, 1.5kb, 2kb, 2.5kb, 3kb, 3.5kb, 4kb, 4.5kb, 5kb, 5.5kb, 6kb, 6.5kb, 7kb, or 7.5kb. In some cases, a large transgene is operably linked to one or more REs, wherein each RE is no more than 49bp, 50bp, 56bp, 60bp, 70bp, 80bp, 90bp, 100bp, 110bp, 117bp, 120bp, 130bp, 140bp, 150bp, 160bp, 170bp, 180bp, 190bp, 200bp, 210bp, 220bp, 230bp, 240bp, 250bp, 259bp, 260bp,

265bp, 270bp, 280bp, 290bp, 300bp, 310bp, 320bp, 330bp, 340bp, 350bp, 360bp, 370bp, 380bp, 390bp, or 400bp, and comprises a sequence according to any one of **TABLE 1** or SEQ ID NOs: 1-2, 13-17, and 22-41. In some cases, the REs increase expression of the transgene by at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 15 fold, at least 20 fold, at least 25 fold, at least 50 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, or at least 1000 fold as an expression vector or cassette without the REs, or as compared to a negative control (e.g., vector alone control or a vector comprising a sequence with no known expression activity).

[00153] When assessing the activity of an expression cassette or vector, the activity or expression can be represented as an activity or expression level per unit dose, or normalized to a dose of expression cassette or vector administered or delivered to a cell, mouse, or a subject. In some cases, expression or activity of a transgene is normalized to an amount of plasmid or DNA (e.g., µg/kg per mouse), or viral particles (e.g., normalized to an amount of genome copies/kg per mouse or subject) used to allow comparison across different expression vectors or cassettes with or without a regulatory element. For example, when assessing a regulatory element's activity in a mouse, transgene expression or transgene activity in cells assayed can be normalized to a dose of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 µg, or greater than 10 µg, or greater than 20 µg of expression vector, cassette, or plasmid per mouse. In some cases, the expression level or activity can be normalized to 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, or 10^{15} gc/kg of viral particles containing an expression vector or cassette as disclosed herein per mouse.

[00154] One advantage of delivering expression cassettes of this disclosure using gene therapy, e.g., rAAV, is that such therapies can provide more targeted and sustained therapeutic effects over time. Additionally, viral gene therapies can be engineered to have tropism for one or more cell types or tissues of interest (e.g., hepatocytes or liver). For example, viral gene therapies can be engineered to infect and deliver a payload or a therapeutic agent, e.g., a transcriptional modulator or a transgene, to one or more regions, tissues, or cell types in vivo.

Transgenes

[00155] The constructs (e.g., expression cassette or vectors) disclosed herein can be used to drive expression of a transgene in a cell or a plurality of cells of interest. In some cases, constructs disclosed herein are used for recombinant protein expression. For example,

regulatory elements disclosed herein can be used to drive expression of recombinant proteins. Recombinant proteins produced with methods of this disclosure can be produced for therapeutic purposes, research purposes, or commercial purposes. Examples of transgenes that can be expressed as part of an in vivo, ex vivo, or in vitro protein expression system include, but are not limited to: Cas9, Factor VIII, DNA binding proteins, hormones, growth/differentiation factors, e.g., insulin, growth hormone, VEGF, neurotrophic factor, fibroblast epithelial factor; therapeutic proteins that regulate or modulate the immune system, e.g., cytokines, interleukins, lymphokines, tumor necrosis factor, antibodies, immunoglobulins, interferons, chimeric T cell receptors; lipoprotein receptor; cystic fibrosis transmembrane regulator; a gene associated with mucopolysaccharidosis types I, II, III, and IV; beta globin; and lipoprotein lipase. In some cases, the transgene expressed is ATP7A, ATP7B, ATP8B1, ABCB4, ABCB11, or a variant or functional fragment thereof. In some cases, the transgene expressed is CDKL5, CNTNAP2, ZEB2, or a variant or functional fragment thereof. In some cases, the transgene is a fibrinogen, prothrombin, a coagulation factor, or a blood clotting factor (e.g., Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12), a variant or functional fragment thereof.

[00156] In some cases, an expression cassette comprising one or more REs of this disclosure operably linked to a transgene is adapted for a gene therapy treatment. In some cases, the gene therapy treatment is administered or adapted for systemic delivery, such as by intravenous infusion or injection. In some cases, the gene therapy is adapted for expression in the liver or hepatocytes. In some cases, the operably linked transgene is a gene that is expressed in the liver or in hepatocytes, or is a gene relevant in a liver disease or condition, e.g, ATP7B associated with Wilson's disease; ABCB4 associated with progressive familial intrahepatic cholestasis type 3; ALDOB associated with hereditary fructose intolerance; GBE1 associated with glycogen storage disease type IV; FAH associated with tyrosinemia type I; ASL associated with argininosuccinate lyase deficiency; SLC25A13 associated with citrin deficiency (CTLN2, NICCD); LIPA associated with cholesteryl ester storage disease; SERPINA1 associated with alpha-1 antitrypsin deficiency; CFTR associated with cystic fibrosis; HFE associated with hereditary hemochromatosis; or ALMS1 associated with Alström syndrome. In some cases, one or more regulatory elements or an expression cassette comprising a RE disclosed herein are used for a gene therapy treatment to treat an inherited liver disease, e.g., a disorder of bile acid synthesis, a disorder of carbohydrate metabolism, a disorder of amino acid metabolism, a urea cycle disorder, or a disorder of lipid metabolism. In some cases, one or more regulatory elements or an expression cassette comprising a RE

disclosed herein are used for gene therapy treatment (e.g., AAV gene therapy) to treat Wilson's disease; progressive familial intrahepatic cholestasis type 3; hereditary fructose intolerance; glycogen storage disease type IV; tyrosinemia type I; argininosuccinate lyase deficiency; citrin deficiency (CTLN2, NICCD); cholesteryl ester storage disease; alpha-1 antitrypsin deficiency; cystic fibrosis; hereditary hemochromatosis; or Alström syndrome. In some cases, one or more regulatory elements or an expression cassette comprising a RE disclosed herein are used for gene therapy treatment (e.g., AAV gene therapy) to treat a blood clotting disorder (e.g., hemophilia, hemophilia A, or hemophilia B) or Wilson's disease.

[00157] In some cases, a method of expressing a recombinant protein comprises introducing an expression cassette or vector into a cell or a plurality of cells, wherein the expression cassette or vector comprises one or more REs of this disclosure (SEQ ID NOs: 1-2, 13-17, and 22-41) operably linked to a transgene. In some cases, the transgene is any one of Cas9, Factor VIII, DNA binding proteins, hormones, growth/differentiation factors, e.g., insulin, growth hormone, VEGF, neurotrophic factor, fibroblast epithelial factor; therapeutic proteins that regulate or modulate the immune system, e.g., cytokines, interleukins, lymphokines, tumor necrosis factor, antibodies, immunoglobulins, interferons, chimeric T cell receptors; lipoprotein receptor; cystic fibrosis transmembrane regulator; a gene associated with mucopolysaccharidosis types I, II, III, and IV; beta globin; and lipoprotein lipase. In some cases, the transgene expressed is ATP7A, ATP7B, ATP8B1, ABCB4, ABCB11, or a variant or fragment thereof. In some cases, the transgene expressed is CDKL5, CNTNAP2, ZEB2, or a variant or fragment thereof. In some cases, the transgene is a fibrinogen, prothrombin, a coagulation factor, or a blood clotting factor (e.g., Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12).

[00158] Nucleotide and protein sequences of human genes disclosed herein can be found in various public databases, including GenBank (NCBI) or UniProKB databases. For examples, human UniProtKB accession numbers include: Q04656 (ATP7A); P35670 (ATP7B); O43520 (AT8B1); P21439 (MDR3, also referred to as ABCB4); O95342 (ABCBB, also referred to as ABCB11); O76039 (CDKL5); Q9UHC6 (CNTP2, also referred to as CNTNAP2); O60315 (ZEB2); P12259 (F5 or Factor V); P00451 (F8 or Factor VIII); P00740 (F9 or Factor IX); P00742 (F10 or Factor X). Any one of such protein sequences can be encoded by a nucleic acid sequence (or a transgene) in an expression cassette disclosed herein.

[00159] Recombinant proteins can be produced in bacterial cells, yeast cells, insect cells, plant cells, or mammalian cells. In some examples, recombinant proteins are produced in mammalian cells. In some cases, proteins are produced in animal models. Examples of cell

lines which can be used to produce proteins include Chinese hamster ovary cells (CHO), mouse myeloma cells (NSO), baby hamster kidney cells (BHK), and B16 melanoma cells. In some examples, human-derived cell lines are used. Examples of human cell lines which can be used to produce proteins include HEK293T, HeLa, HT-1080, and PER.C5. Recombinant proteins can also be produced in animals or in animal products. Recombinant proteins can also be produced in plants. Recombinant proteins may be expressed in the leaves, stems, flowers, fruits or seeds of a plant.

[00160] In some embodiments, a regulatory element of the disclosure can be used to drive or increase expression of a reporter gene, such as luciferase. In some embodiments, one or more regulatory elements can result in expression of an operably linked luciferase transgene at levels that yield an output of more than 10^{17}, 10^{18}, 10^{19}, 10^{20}, or 10^{21} photons/sec/mouse as measured according to the method of Example 2.

[00161] Also contemplated herein is a method of treating a haploinsufficiency or any disorder or condition associated with insufficient gene expression and/or protein activity or synthesis (or under-expression of a gene), comprising administering an expression cassette or vector in a subject in need thereof, wherein the expression cassette or vector comprises one or more REs of this disclosure (SEQ ID NOs: 1-2, 13-17, and 22-41) operably linked to a transgene. In some cases, the transgene is a DNA binding protein or a transcriptional modulator (e.g., a transcriptional activator) that results in increased expression of an endogenous gene. In some cases, the transgene in the expression cassette or vector is a therapeutic transgene. In some cases, the therapeutic transgene is any one of Cas9, Factor VIII, DNA binding proteins, hormones, growth/differentiation factors, e.g., insulin, growth hormone, VEGF, neurotrophic factor, fibroblast epithelial factor; therapeutic proteins that regulate or modulate the immune system, e.g., cytokines, interleukins, lymphokines, tumor necrosis factor, antibodies, immunoglobulins, interferons, chimeric T cell receptors; lipoprotein receptor; cystic fibrosis transmembrane regulator; a gene associated with mucopolysaccharidosis types I, II, III, and IV; beta globin; and lipoprotein lipase. In some cases, the transgene expressed is ATP7A, ATP7B, AT8B1 (or ATP8B1), MDR3 (or ABCB4), ABCBB (or ABCB11), CDKL5, CNTP2 (or CNTNAP2), ZEB2, Factor V, Factor VIII, Factor IX, or Factor X, or a variant, subunit, isoform, or functional fragment thereof. Progressive familial intrahepatic cholestasis (PFIC) includes PFIC1, PFIC2, and PFIC3, which are associated with mutation(s) in ATP8B1, ABCB11, and ABCB4 genes. Progressive familial intrahepatic cholestasis type 2 (PFIC2) is associated with a defect or a mutation in ABCB11. ATP8B1 gene mutations can cause PFIC1. Mutations in the ABCB11 gene can

cause PFIC2. ABCB4 gene mutations can cause PFIC3. In some cases, a transgene disclosed herein is CDKL5 and is used in a gene therapy to treat CDKL5 deficiency disorder. A mutation or defect in CNTNAP2 can result in autism spectrum disorder. In some cases, the transgene is a fibrinogen, prothrombin, a coagulation factor, or a blood clotting factor (e.g., Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12), or a variant or functional fragment thereof. In some cases, the transgene is expressed preferentially in hepatocytes. In some cases, the administering is systemic, e.g., via oral, intravenous, intramuscular, intraperitoneal, intrathecal, enteral, or parenteral administration. In some cases, the method comprises delivering the expression cassette or vector via intravenous infusion or injection. In some cases, the administering is local, e.g., via injection into the liver, CNS, or an organ in a body. In some cases, the expression cassette or vector is an AAV, e.g., AAV8.

[00162] In some cases, a method of treating a disorder or condition associated with overexpression of a gene is contemplated herein, the method comprising administering an expression cassette or vector into a subject in need thereof, wherein the expression cassette or vector comprises one or more REs of this disclosure (SEQ ID NOs: 1-2, 13-17, and 22-41) operably linked to a transgene. In some cases, the transgene is a DNA binding protein or a transcriptional modulator (e.g., a transcriptional repressor) that results in decreased expression of an endogenous gene. In some cases, the transgene is expressed preferentially in hepatocytes. In some cases, the administering is systemic, e.g., via oral, intravenous, intramuscular, intraperitoneal, intrathecal, enteral, or parenteral administration. In some cases, the method comprises delivering the expression cassette or vector via intravenous infusion or injection. In some cases, the administering is local, e.g., via injection into the liver, CNS, or an organ in a body. In some cases, the expression cassette or vector is an AAV, e.g., AAV8.

[00163] In some cases, a method of treating a genetic disorder or condition associated with a defect in any one of the following genes is contemplated herein: ATP7A, ATP7B, AT8B1 (or ATP8B1), MDR3 (or ABCB4), ABCBB (or ABCB11), CDKL5, CNTP2 (or CNTNAP2), ZEB2, Factor V, Factor VIII, Factor IX, Factor X, a fibrinogen, prothrombin, a coagulation factor, or a blood clotting factor (e.g., Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12). In some cases, disclosed herein is a method of treating Wilson's disease. In some cases, the method of treating Wilson's disease or any other genetic disorder or condition associated with ATP7A, ATP7B, AT8B1 (or ATP8B1), MDR3 (or ABCB4), ABCBB (or ABCB11), CDKL5, CNTP2 (or CNTNAP2), ZEB2, Factor V, Factor VIII, Factor IX, Factor X, a fibrinogen, prothrombin, a coagulation factor, or a blood clotting factor (e.g., Factor 1, 2, 3, 4, 5, 6, 7, 8,

9, 10, 11, or 12) comprises administering an expression cassette or vector disclosed herein into a subject in need thereof (e.g., an animal or a human), wherein the expression cassette or vector comprises one or more REs of this disclosure operably linked to a transgene of this disclosure, e.g., ATP7A, ATP7B, AT8B1 (or ATP8B1), MDR3 (or ABCB4), ABCBB (or ABCB11), CDKL5, CNTP2 (or CNTNAP2), ZEB2, Factor V, Factor VIII, Factor IX, Factor X, a fibrinogen, prothrombin, a coagulation factor, a blood clotting factor (e.g., Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12), a DNA binding protein, or a transcriptional modulator. In some cases, the transgene is a Cas protein (e.g., Cas9 or saCas9 protein) and/or a guide RNA that edit an endogenous gene (e.g., a Cas removes a mutation from an endogenous gene). In some cases, the expression cassette or vector expresses the transgene preferentially in the liver. In some cases, the administering is systemic, e.g., via intravenous infusion or injection. In some cases, the administering is local, e.g., injection directly into a tissue or organ, e.g., injection into the liver or CNS.

[00164] In some cases, an expression cassette or vector disclosed herein is used to treat Wilson's disease, wherein the expression cassette or vector comprises one or more REs (e.g., SEQ ID NO: 1, 2, 3, 4, 13, 14, 15, 16, 17, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41) operably linked to a therapeutic transgene, wherein the transgene is ATP7B or a transcriptional modulator or a DNA binding protein that acts on an endogenous ATP7B gene to increase ATP7B expression in a cell or in vivo. In some cases, such expression cassette or vector is delivered into a subject diagnosed with or at risk for Wilson's disease. In some cases, such expression cassette or vector is delivered into a subject via intravenous infusion or injection. In some cases, the delivery is systemic. In some cases, a method of treating Wilson's disease comprising administering a gene therapy (e.g., an AAV gene therapy) into a subject in need thereof, wherein the gene therapy comprises one or more REs (e.g., SEQ ID NO: 1, 2, 3, 4, 13, 14, 15, 16, 17, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41) operably linked to a therapeutic transgene, wherein the transgene is ATP7B or a transcriptional modulator that acts on an endogenous ATP7B gene to increase ATP7B expression in a cell or in vivo. In some cases, the expression cassette or vector is an AAV, e.g., AAV8. In some cases, the expression cassette or vector is preferentially expressed in hepatocytes or in the liver.

[00165] In some cases, an expression cassette or vector disclosed herein is used to treat a blood clotting disorder, wherein the expression cassette or vector comprises one or more REs (e.g., SEQ ID NO: 1, 2, 3, 4, 13, 14, 15, 16, 17, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41) operably linked to a therapeutic transgene, wherein the

transgene is a fibrinogen, prothrombin, a coagulation factor, or a blood clotting factor (e.g., Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12) or a transcriptional modulator that acts on an endogenous gene corresponding to a fibrinogen, prothrombin, a coagulation factor, or a blood clotting factor (e.g., Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12). In some cases, the transgene is Factor VIII. In some cases, such expression cassette or vector is delivered into a subject diagnosed with or at risk for a blood clotting disorder. In some cases, such expression cassette or vector is delivered into a subject via intravenous infusion or injection. In some cases, the delivery is systemic. In some cases, a method of treating a blood clotting disorder comprising administering a gene therapy (e.g., an AAV gene therapy) into a subject in need thereof, wherein the gene therapy comprises one or more REs of this disclosure (e.g., SEQ ID NO: 1, 2, 3, 4, 13, 14, 15, 16, 17, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41) operably linked to a therapeutic transgene, wherein the transgene is Factor VIII or a transcriptional modulator that acts on an endogenous Factor VIII gene to increase its expression in a cell or in vivo. In some cases, the transgene is any one of Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12. In some cases, the expression cassette or vector is an AAV. In some cases, the expression cassette or vector is preferentially expressed in hepatocytes or in the liver.

[00166] In some cases, the transgene may be a DNA binding protein, or a protein associated with DNA binding. In some cases, a DNA binding protein may be a transcription factor, or a part of a transcription factor. In some cases, a DNA binding protein may be Cas9, a Cas family protein, saCas9, dCas9, a dCas family protein, a transcriptional activator like effector (TALE) protein, or a zinc finger protein, or a fusion protein. In some cases, a method of treating a genetic disorder comprises administering an expression cassette or vector comprising a gene editing protein (e.g., Cas9 and a guide RNA) and one or more REs of this disclosure (e.g., SEQ ID NO: 1, 2, 3, 4, 13, 14, 15, 16, 17, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41). In some cases, the guide RNA targets the Cas protein to an endogenous gene of interest or an endogenous gene disclosed herein.

[00167] In some cases, the constructs disclosed herein are used to deliver a gene therapy. In particular, relatively short regulatory elements of this disclosure can be of particular utility for gene therapy requiring the expression of large transgenes, such as Factor VIII or ATP7B. In some embodiments, a large transgene is expressed in liver cells, such as hepatocytes. In other embodiments, a large transgene is expressed in central nervous system (CNS), such as neurons. In some embodiments, any one of the transgenes disclosed herein is operably linked to one or more regulatory elements disclosed herein to increase expression of the transgene.

In some cases, transgenes disclosed herein are expressed in a cell or in vivo, in vitro, and/or ex vivo. In some cases, such large transgenes are delivered into a cell using a gene therapy, a recombinant expression vector or plasmid, or a viral vector or plasmid. Examples of large transgenes that can be expressed as part of a gene therapy or an expression system ex vivo or in vitro include, but are not limited to: Menkes' protein (ATP7A), ATP binding cassette subfamily b member 4 (ABCB4), ATPase phospholipid transporting 8B1 (ATP8B1), ATPase copper transporting beta (ATP7B), cyclin-dependent kinase-like 5 (CDKL5), contactin associated protein-like 2 (CNTNAP2), ATP binding cassette subfamily B member 11 (ABCB11), and zinc finger E-box-binding homeobox 2 (ZEB2), and any variant, subunit, mutant, or functional fragment thereof, including codon-optimized variants.

[00168] In some embodiments, the constructs disclosed herein are used for gene editing. For example, a construct can comprise a regulatory element as disclosed herein, a Cas9 coding sequence, and a guide RNA. In some cases, the Case9 coding sequence encodes saCas9. A construct with a Cas9 or saCas9 coding sequence and guide RNA can be used to correct a dysfunctional gene, for example, by correcting a mutation (e.g., removing a premature stop codon).

[00169] Also contemplated herein are gene therapies or expression vectors comprising one or more regulatory elements as disclosed herein to treat various diseases, including various genetic disorders, such as severe combined immune deficiency (ADA-SCID), Menkes disease, familial intrahepatic cholestasis, Byler syndrome, Wilson disease, cortical dysplasia-focal epilepsy syndrome, Mowat-Wilson syndrome, PFIC2, PFIC3, chronic granulomatous disorder, hemophilia, congenital blindness, metabolic disorders, lysosomal storage disease, muscular dystrophy, cancer, viral infections, heart diseases, and diabetes. In some cases, a gene therapy comprising one or more regulatory elements as disclosed herein may be used to treat Wilson's disease.

[00170] In some embodiments, the regulatory elements disclosed herein are used to treat a subject with a blood clotting disorder. For example, an expression cassette containing a regulatory element of this disclosure and a coding sequence for FVIII can be used to rescue a clotting disorder or disease in a subject with a FVIII deficiency. Transfecting an expression cassette containing a regulatory element of this disclosure and a coding sequence for FVIII into a subject with a clotting disorder may result in a decrease in blood loss after injury, as shown in **FIG. 5A** and **FIG. 10**. Transfecting an expression cassette containing a regulatory element of this disclosure and a coding sequence for FVIII into a subject with a clotting disorder may result in a blood loss of at least about 10%, 20%, 30% Transfecting an

expression cassette containing a regulatory element of this disclosure and a coding sequence for FVIII into a subject with a clotting disorder may result in a decrease time for bleeding to stop after injury, as shown in **FIG. 5B** and **FIG. 11**.

[00171] Subjects that can be treated with any one of the constructs, gene therapy, or expression vectors/cassettes of this disclosure include humans, primates, dogs, cats, rodents, horses, cows, sheep, pigs, and other livestock. In some cases, the subject has a disease/disorder or is at risk of a disease/disorder. For example, the subject may have a genetic disease, or have a DNA sequence known to be linked to a genetic disease or predisposition for the disease. In some cases, the subject has hemophilia. In some cases, the subject has hemophilia A. In some embodiments, the subject has a defect in Factor VIII. In some embodiments, a gene therapy comprising one or more regulatory elements described herein operably linked to Factor VIII is used to treat a subject diagnosed with hemophilia A.

EXAMPLES

[00172] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

EXAMPLE 1

High Expression in HEK293T Cells

[00173] HEK293T cells were transfected with plasmid DNA containing a luciferase gene under the control of one of several different regulatory elements, i.e., no promoter control, SCP, CMV, SEQ ID NO: 3 (SEQ ID NO: 1 operably linked to minCMV), SEQ ID NO: 4 (SEQ ID NO: 2 operably linked to minCMV), and CAG. The normalized luciferase values from each construct are illustrated in **FIG. 1A**. The size-normalized activity values from each construct are illustrated in **FIG. 1B**. The sequences of the regulatory elements and promoters used herein are provided in **TABLE 1** above and **TABLE 3** below. Regulatory element SEQ ID NO: 1 linked to a minCMV promoter and regulatory element SEQ ID NO: 2 linked to a minCMV promoter drove higher levels of luciferase expression than minCMV alone and SCP alone. Both SEQ ID NO: 1 and SEQ ID NO: 2, when linked to a minCMV promoter, drove high expression of luciferase in HEK293T kidney cells as compared to the control, SCP, or minCMV promoter without the regulatory element (i.e., SEQ ID NO: 1 or 2).

[00174] Similar normalized luciferase expression experiments were done with additional controls and regulatory sequences, as illustrated in **FIG. 1C, FIG. 1D**, and **FIG. 1E**. Firefly expression was also assayed to ensure similar transfection efficiency in all the samples tested.

In **FIG. 1C**, normalized luciferase expression of regulatory elements SEQ ID NO: 4, SEQ ID NOs: 17 and 22 combined (or SEQ ID NO: 17/22), SEQ ID NOs: 16 and 23 combined (or SEQ ID NO: 16/23), SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33 were compared with two negative controls (i.e., sequences known to not drive any expression). Each of the regulatory elements tested resulted in high levels of luciferase expression, e.g., by at least 10, 50, or 100 or more fold as compared to the negative controls.

[00175] In **FIG. 1D**, normalized luciferase expression from plasmids comprising regulatory elements SEQ ID NO: 4, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 26, or SEQ ID NO: 27 were compared to a negative control, and a similar plasmid comprising either CMV linked to CMVe or CMV operably linked to luciferase. Each regulatory element tested (i.e., SEQ ID NO: 4, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 26, and SEQ ID NO: 27) resulted in higher luciferase expression than the negative control, CMV alone, and CMV+CMVe. The relatively short REs tested showed at least 10 fold, at least 15 fold, at least 20 fold, at least 30 fold, at least 40 fold, or more than 50 fold normalized luciferase expression as compared to a plasmid comprising a CMV promoter or CMV+CMVe linked to luciferase.

[00176] In **FIG. 1E**, normalized luciferase expression from plasmids comprising regulatory elements SEQ ID NO: 17/22, SEQ ID NO: 16/23, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, or SEQ ID NO: 41 operably linked to luciferase were compared to a negative control (i.e., sequence known to have no expression activity). Each regulatory element (i.e., SEQ ID NOs: 17/22, 16/23, and 34-41) drove higher luciferase expression than the negative control, while each of SEQ ID NO: 35 and SEQ ID NO: 39 drove a normalized luciferase expression higher than 10^2, or at least 100 fold higher luciferase expression than the negative control.

[00177] TABLE 3: Additional Sequences Disclosed Herein

SEQ ID NO.	Sequence (5'-to-3')	Name
5	GTACTTATATAAGGGGGTGGGGGCGCGTTCGTCCTCAGTCGCGA TCGAACACTCGAGCCGAGCAGACGTGCCTACGGACC	SCP
6	GGGGAGGCTGCTGGTGAATATTAACCAAGGTCACCCCAGTTATC GGAGGAGCAAACAGGGGCTAAGTCCACGCTAGCGTCTGTCTGCA CATTTCGTAGAGCGAGTGTTCCGATACTCTAATCTCCCTAGGCAA GGTTCATATTTGTGTAGGTTACTTATTCTCCTTTTGTTGACTAAGT CAATAATCAGAATCAGCAGGTTTGGAGTCAGCTTGGCAGGGATC AGCAGCCTGGGTTGGAAGGAGGGGGTATAAAAGCCCCTTCACCA GGAGAAGCCGTC	SerpE_TTR
7	GTTTGCTGCTTGCAATGTTTGCCCATTTTAGGGTGGACACAGGAC GCTGTGGTTTCTGAGCCAGGGCTAGCGGGCGACTCAGATCCCAG CCAGTGGACTTAGCCCCTGTTTGCTCCTCCGATAACTGGGGTGAC	Proto1

SEQ ID NO.	Sequence (5'-to-3')	Name
	CTTGGTTAATATTCACCAGCAGCCTCCCCCGTTGCCCCTCTGGAT CCACTGCTTAAATACGGACGAGGACAGGGCCCTGTCTCCTCAGC TTCAGGCACCACCACTGACCTGGGACAGTGAATCGCCAC	
8	TGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTT GACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGG GAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTC GTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTA CGGTGGGAGGTCTATATAAGCAGAGCT	minCMV
9	GTTTGCTGCTTGCAATGTTTGCCCATTTTAGGGTGGACACAGGAC GCTGTGGTTTCTGAGCCAGGGGGCGACTCAGATCCCAGCCAGTG GACTTAGCCCCTGTTTGCTCCTCCGATAACTGGGGTGACCTTGGT TAATATTCACCAGCAGCCTCCCCCGTTGCCCCTCTGGATCCACTG CTTAAATACGGACGAGGACAGGGCCCTGTCTCCTCAGCTTCAGG CACCACCACTGACCTGGGACAGTGAATC	UCL-HLP
10	CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCA ACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATA GTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTA TTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATAT GCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG CCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTT GGCAGTACATCTACGTATTAGTCATCGCTATTACCATG	CMVe
11	GTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAA CGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGT AACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATT TACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATG CCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTG GCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGG TGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCC ACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCG ATGGGGGCGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGG CGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGG CAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCG AGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGC GGGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGC GCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTC CCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAA TTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGT GAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGA GCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCC GCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGC GGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCG GCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAA CAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGG GGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCCCCTGCACCCCC CTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTC CGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGT GGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGC CGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCGGAGCGCCG GCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGG TAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTG GCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGG	CAG

SEQ ID NO.	Sequence (5'-to-3')	Name
	GCGCGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCG GGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCATC TCCAGCCTCGGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGG GACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGC TCTAGAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTTCCTAC AGCTCCTGGGCAACGTGCTGGTTGTTGTGCTGTCTCATCATTTTG GCAAAGAATT	
12	GCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTC CCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTA GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACT GGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGT GCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCC AGAACACAGG	EFS

EXAMPLE 2

Global High Drivers

[00178] To assess the expression patterns of regulatory elements SEQ ID NO: 1 and SEQ ID NO: 2, recombinant AAV vectors were made containing each regulatory element linked to luciferase. Regulatory elements used were: SCP, SERpE_TTR, Proto1, CMV, UCL-HLP, CMVe, SEQ ID NO: 1, and SEQ ID NO: 2. Plasmid DNA was delivered to SCID-beige mice (12 µg of expression vector per mouse) via hydrodynamic tail vein injection and expression of luciferase was assayed by injecting the mice with 5 ug (~ 0.25 mg/kg) of furimazine 48 hours post injection**. FIG. 2A** illustrates the expression of luciferase under the control of different regulatory elements, and illustrates SEQ ID NOs: 1 and 2, when each RE is linked to minCMV, were able to drive expression of the operably linked transgene in at least one additional cell type as well as kidney cells, as illustrated by the white regions indicative of luciferase expression in various tissues in mice. As illustrated in **FIG. 2B**, regulatory elements SEQ ID NO: 3 and SEQ ID NO: 4 each resulted in high levels luciferase expression as compared to the other regulatory elements, as measured by luciferase luminescence in photons/sec.

EXAMPLE 3

Expression of Human Factor VIII

[00179] SCID-beige mice were injected with 16 µg of expression cassette DNA containing a coding sequence for Factor VIII (NIH Protein Accession ID ABV90867.1) operably linked with UCL-HLP; minCMV with SEQ ID NO: 1; or minCMV with SEQ ID NO: 2. At least eight mice were injected with each of the 3 plasmids, and at least an additional eight mice were injected with buffer. 24h after injection with plasmid DNA, blood samples were taken

and blood plasma was analyzed for Factor VIII activity and concentration using established methods (COATEST® SP4 FVIII, Chromogenix; Visualize™ DVIII Antigen Kit, Affinity Biologicals INC.). As illustrated in **FIG. 3A**, both minCMV with SEQ ID NO: 1 and minCMV with SEQ ID NO: 2 expression cassettes resulted in high plasma concentrations of Factor VIII at levels of 1.5 IU/ml or higher. **FIG. 3B** illustrates that both SEQ ID NO: 3 (minCMV with SEQ ID NO: 1) and SEQ ID NO: 4 (minCMV with SEQ ID NO: 2) resulted in plasma Factor VIII activity levels of 100% or higher. Regulatory elements SEQ ID NOs: 1 and/or 2 when operably linked to Factor VIII coding sequence together with minCMV resulted in blood Factor VIII activity levels of 114% and 166%, respectively, which were about 10 fold and 15 fold higher than the activity seen with a UCL-HLP promoter.

EXAMPLE 4

Gene Editing

[00180] An all-in-one recombinant AAV virion was developed to delete a stop cassette upstream of tdTomato in transgenic mice Ai9 (Jax Stock No: 007909). Once the stop cassette is deleted by saCas9, tdTomato is expressed under the control of a CAG promoter. rAAVs were developed containing saCas9 under the control of either a EFS promoter, a CMV promoter, or a CMV promoter with SEQ ID NO: 2. The rAAVs further comprised two Sa guide RNA scaffolds under the control of U6 promoters. These rAAV virions were injected into the Dente Gyrus of the transgenic mice described above. Brains were fixed and sectioned after 3 weeks, and expression of tdTomato in the dente gyrus was assessed. As illustrated in **FIG. 4B**, CMV with SEQ ID NO: 2 (or SEQ ID NO: 4) resulted in more tdTomato expressing cells than CMV alone or EFS. **FIG. 4A** illustrates that SEQ ID NO: 2 when linked to CMV (or SEQ ID NO: 4) drove expression of the linked transgene (RFP) in at least a subpopulation of brain cells, e.g., neurons.

EXAMPLE 5

Gene Therapy Treatment of a Blood Clotting Disorder

[00181] Genetically engineered FVIII knockout mice (F8tm1Srcmo, deletion of Exon 16-19 of F8 gene; obtained from Shanghai Model Organism Center, Inc., Shanghai, China) have a clotting deficiency which can be assayed by measuring either the volume of blood released by clipping the end of the tail and allowing it to bleed out till clotting occurs, or the time elapsed till clotting occurs. FVIII knockout mice were injected with phosphate buffered saline (PBS control) or recombinant AAV virions containing SEQ ID NO: 4 driving

expression of a FVIII transgene. The rAAV virions were injected at two different doses: 3^{11} gc/kg or 3^{12} gc/kg. Three weeks after the injections, the treated FVIII-knockout mice were tail clipped and time and blood volume loss till clotting were measured. A group of non-knockout mice were also tail clipped and assessed for blood volume and time till clotting. As shown in **FIG. 5A**, the PBS treated FVIII knockout mice lost more than double the volume of blood than the untreated non-knockout mice. However, the rAAV treated FVIII knockout mice lost less blood than the PBS treated mice. At the higher rAAV dose, the treated mice lost the same blood volume as the untreated non-knockout mice. Similar results were seen for the bleeding time assay, as illustrated in **FIG. 5B**. While the lower rAAV dose did not show an effect on bleeding time, the higher dose of rAAV containing SEQ ID NO: 4 operably linked to FVIII reduced the bleeding time almost to the time for the non-knockout mice.

EXAMPLE 6

Gene Therapy Treatment of a Blood Clotting Disorder

[00182] FVIII knockout mice (as described in Example 5) were injected with phosphate buffered saline or recombinant AAV virions containing each of SEQ ID NOs: 13-17 driving the expression of FVIII, or a control virion containing SEQ ID NO: 9 (UCL-HLP) driving the expression of FVIII. **FIG. 8** illustrates the concentrations of FVIII in blood three weeks after the transfection for untreated knockout mice (KO) and the mice treated with the various virions. The virions containing SEQ ID NO 13, SEQ ID NO: 14, or SEQ ID NO: 17 resulted in the highest expression of FVIII in vivo; SEQ ID NO: 15 resulted in moderate expression of FVIII as compared to the other regulatory elements; and SEQ ID NO: 16 resulted in low expression of FVIII as compared to the other sequences. However, SEQ ID NO: 16 still resulted in FVIII concentration of about 2 fold higher than with the UCL-HLP promoter.

[00183] Activity of the expressed FVIII was also assayed, as described in Example 3, as illustrated in **FIG. 9** and **TABLE 4**. Activity levels from the different virions followed the same pattern as the expression levels.

[00184] TABLE 4: Activity of FVIII in Blood of Transfected Mice

	KO	SEQ ID NO: 13	SEQ ID NO: 14	SEQ ID NO: 15	SEQ ID NO: 16	SEQ ID NO: 17	SEQ ID NO: 9
Activity level of FVIII (% of Wildtype) in the different mice	-1.4019	-1.1497	172.7369	19.9399	1.0077	5.2719	-0.7564
	-1.412	93.1133	46.0407	14.038	0.8576	6.1928	-0.4091
	-1.0884	62.7729	198.6102	3.0212	0.8175	20.677	-0.2897
	-1.9783	2.2538	4.9381	9.5587	0.6174	23.1995	-0.7022
	-2.1097	86.574	6.5522	12.6458	2.6694	12.9859	-0.8216
	-1.8427	14.8857	35.0801	4.5446	3.4701	154.5709	-0.4308
	-1.9392	64.9174	13.806	12.3936	2.0187	53.9879	0.3508

	KO	SEQ ID NO: 13	SEQ ID NO: 14	SEQ ID NO: 15	SEQ ID NO: 16	SEQ ID NO: 17	SEQ ID NO: 9
	-1.6585	12.4836	31.4121	2.9909	-0.8641	15.8733	-0.7347
	-1.5796	1.2449	26.6502	18.2246	-0.794	44.1145	-0.5285
			1.2355	8.8654	1.0177	63.181	-0.192
Average	**-1.6678**	**37.4551**	**53.7062**	**10.62227**	**1.0818**	**40.00547**	**-0.45142**

[00185] Three weeks after the injections, the treated FVIII knockout mice were tail clipped and time and blood volume till clotting were measured. A group of non-knockout mice were also tail clipped and assessed for blood volume and time till clotting. As illustrated in **FIG. 10** and **TABLE 5**, the PBS treated FVIII knockout mice lost more than double the volume of blood than the untreated non-knockout mice (or WT mice). However, the rAAV treated FVIII knockout mice lost less blood than the PBS treated mice. The mice which received the virion containing SEQ ID NO: 17 lost similar blood volume as the untreated non-knockout mice. Similar results were observed for the bleeding time assay, as shown in **FIG. 11** and **TABLE 6**. The bleeding time for mice treated with virions comprising SEQ ID NO: 15 showed the strongest reduction in bleeding time and blood loss.

[00186] TABLE 5: Blood Volume Lost Before Clotting in Mice Transfected With Different Virions

	KO	SEQ ID NO: 13	SEQ ID NO: 14	SEQ ID NO: 15	SEQ ID NO: 16	SEQ ID NO: 17	SEQ ID NO: 9	WT
Blood volume lost from different mice (g)	0.0814	0.3264	0.1107	0.0223	0.3606	-0.0114	0.5145	0.0447
	0.4122	0.05	0.1479	0.1471	0.1367	0.0145	1.1232	0.0001
	-0.0522	0.6822	0.2897	0.0136	0.6372	0.0341	0.4819	-0.0357
	0.6397	0.0178	0.0526	0.0449	0.219	0.0575	0.486	0.0836
	0.3505	0.0356	0.2819	0.3934	0.2606	0.0963	0.4708	0.1065
	0.4982	0.1057	0.1665	0.0331	0.201	0.0837	0.0806	0.0693
	-0.0116	0.2129	0.0934	0.0919	0.0827	0.0288	0.34	0.061
	0.1827	0.0133	0.197	0.0748	0.438	0.103	0.2327	0.0423
	0.2793	0.0573	0.0355	0.0782	0.0275	0.0223	0.4931	0.0919
			0.1657	0.0456	0.1477	0.191	0.0281	0.1329
Average	**0.264**	**0.167**	**0.154**	**0.094**	**0.251**	**0.062**	**0.425**	**0.060**

[00187] TABLE 6: Bleeding Time Till Clotting for Mice Transfected With Different Virions

	KO	SEQ ID NO: 13	SEQ ID NO: 14	SEQ ID NO: 15	SEQ ID NO: 16	SEQ ID NO: 17	SEQ ID NO: 9	WT
Bleeding time for different mice, (mins)	6.42	13.25	3.28	6.78	11.22	2.38	27.63	4.55
	25.23	8.3	11.02	9.5	15.6	1.95	26.88	0.38
	0	28.4	14.78	1.28	24.27	18.93	20.4	0.63
	26.17	0.92	2.52	3.62	27.5	17.73	20.2	5.15
	19.98	4.22	19.02	13.28	19.17	6.7	30	4.6
	30	16.95	3.27	1.15	23.5	9.95	13.15	2.13

	KO	SEQ ID NO: 13	SEQ ID NO: 14	SEQ ID NO: 15	SEQ ID NO: 16	SEQ ID NO: 17	SEQ ID NO: 9	WT
	14.65	30	6.38	14.1	13.23	3.87	29.67	3.7
	21.93	7.58	8.17	1.58	22.45	21.43	14.43	4.68
	22.35	17.82	4.73	11.52	12.43	8.5	30	2.33
			14.6	0.35	13.32	13.33	1.66	8.63
Average	**18.52**	**14.16**	**8.13**	**6.97**	**18.81**	**10.16**	**23.59**	**3.12**

EXAMPLE 7

ATP7B Expression in Liver

[00188] C57BL6/J mice were intravenously injected with AAV8 vectors containing ATP7B under the control of SEQ ID NO: 13. Two weeks after viral delivery, livers were harvested. Liver punches were disrupted and homogenized in 600ul of RLT supplemented with 10ul/mL beta-mercaptoethanol, and RNA was extracted using an RNeasy Mini kit (Qiagen) in accordance with manufacturer's protocols, including on-column DNase treatment. For each sample, RNA (3ug) was reverse transcribed with Superscript IV (Invitrogen), using OligoDT primers (65°C 5m, 55°C 10m, 85°C 10m). Primers against human ATP7B (5'-CATTCCAGGACTGTCCATTCT-3'(SEQ ID NO: 18); 5'-GGCCTGAACGTAGAAGTACCA-3' (SEQ ID NO: 19)), and GAPDH (5'ACCACAGTCCATGCCATCAC-3' (SEQ ID NO: 20); 5'-TCCACCACCCTGTTGCTGTA-3' (SEQ ID NO: 21)) were verified with standard curves to ensure reliability, and used for qPCR (Phusion, SYBR Green) under the following amplification conditions: (98°C 2m, 40X[98°C 10s, 67°C 30s, 92°C 15s]). ATP7B expression was normalized to GAPDH and presented as fold change over baseline using the delta-delta Ct method. As illustrated in **FIG: 12**, and **TABLE 7**, SEQ ID NO: 13 drove expression of ATP7B in the liver at three different doses, and showed a dose response with the highest viral dose resulting in the highest ATP7B expression.

[00189] TABLE 7: ATP7B Expression in Liver of Transfected Mice

	Buffer	SEQ ID: 13; 1E10 gc/mouse	SEQ ID: 13; 1E11 gc/mouse	SEQ ID: 13; 1E12 gc/mouse
Relative ATP7B expression in liver of transfected mice (log2)	3.214475452	111.7850412	87.30041862	5639.103082
	1.992504985	7.333913922	375.1308235	6142.385453
	2.857162979	20.64780715	306.8202728	1207.591395
	3.20705701	27.24494487	183.2814118	4273.64098
	0.339445061	1.698879272	3.813855015	2376.435176
	0.45520343	0.501590988	2.734448707	1352.347313
	0.405540152		19.17626705	26577.47941
	0.271919214		0.5721968	128.407293
Average	**1.592913535**	**28.20202957**	**122.3537118**	**5962.17376**

[00190] Example 7 illustrates expression cassettes comprising one or more REs disclosed herein operably linked to a ATP7B transgene can be used to treat a disorder or condition associated with ATP7B deficiency, e.g., Wilson's disease, in an animal, a mammal, or a human subject in need thereof. Further, such expression cassettes can be delivered systemically in vivo using rAAV8, e.g., via intravenous injection or infusion.

EXAMPLE 8

High EGFP Transcription in Liver

[00191] C57BL6/J mice were intravenously injected with AAV8 vectors containing EGFP under the control of various REs: SEQ ID NO: 4, 13, 14, 15, 16, 17, and 27. Each AAV8 vector was administered at a dose of $5x10^{11}$ gc/mouse. Two weeks after viral delivery, livers were harvested. Liver punches were disrupted and homogenized in 600ul of RLT supplemented with 10ul/mL beta-mercaptoethanol, and RNA was extracted using an RNeasy Mini kit (Qiagen) in accordance with manufacturer's protocols, including on-column DNase treatment. For each sample, RNA (3ug) was reverse transcribed with Superscript IV (Invitrogen), using OligoDT primers (65 °C 5m, 55 °C 10m, 85 °C 10m). Primers against EGFP (5'- GCTACCCCGACCACATGAAG -3' (SEQ ID NO: 42); 5'-TCTTGTAGTTGCCGTCGTCC -3' (SEQ ID NO: 43), and GAPDH (SEQ ID NO: 20 and SEQ ID NO: 21) were used for qPCR (Phusion, SYBR Green) under the following amplification conditions: (98 °C 2m, 40X[98 °C 10s, 65 °C 30s, 92 °C 15s]). EGFP expression was normalized to GAPDH and presented as fold change over baseline using the delta-delta Ct method (**FIG. 13**). As illustrated in **FIG. 13**, each of the REs tested increased EGFP expression, as measured by RNA transcripts, in the liver. Further, SEQ ID NO: 4, SEQ ID NO: 27, and SEQ ID NO: 14 resulted in comparable levels of EGFP.

[00192] Example 8 illustrates that the relatively short REs of this disclosure can results in high levels of transgene expression in the liver, as measured by RNA transcripts.

[00193] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope

of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

[00194] Various embodiments of this disclosure are defined with reference to the following numbered clauses.

1. An expression cassette comprising a regulatory element operably linked to a therapeutic transgene, wherein the regulatory element comprises one or more of (i) SEQ ID NOs: 1-2, 13-17, and 22-41; or (ii) sequences having at least 80%, 85%, 90%, or 95% sequence identity to any one of (i).
2. The expression cassette of clause 1, wherein the regulatory element is non-naturally occurring.
3. The expression cassette of any one of clauses 1-2, wherein the regulatory element comprises an intronic sequence.
4. The expression cassette of any one of clauses 1-3, wherein the regulatory element is located between a promoter and the therapeutic transgene.
5. The expression cassette of any one of clauses 1-2, wherein the regulatory element comprises a promoter sequence.
6. The expression cassette of clause 5, wherein the regulatory element is the only promoter in the cassette.
7. The expression cassette of any one of clauses 1-6, wherein the regulatory element is relatively short.
8. The expression cassette of any one of clauses 1-6, wherein the regulatory element is no more than 100bp.
9. The expression cassette of any one of clauses 1-8, wherein the regulatory element is no more than 60bp.
10. The expression cassette of any one of clauses 1-9, wherein the regulatory element is no more than 50bp.
11. The expression cassette of any one of clauses 1-10, wherein the expression cassette is part of a rAAV.
12. The expression cassette of clause 11, wherein the rAAV is rAAV8.
13. The expression cassette of any one of clauses 1-10, wherein the expression cassette is part of an adenovirus.
14. The expression cassette of any one of clauses 1-10, wherein the expression cassette is part of a lentivirus.

15. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is larger than 1kb, 1.5kb, 2kb, 2.5kb, 3kb, 3.5kb, 4kb, 4.5kb, 5kb, 5.5kb, 6kb, 6.5kb, 7kb, or 7.5kb.
16. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is ATP7B, or a variant or a functional fragment thereof.
17. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is ATP7A, or a variant or a functional fragment thereof.
18. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is ATP8B1, or a variant or a functional fragment thereof.
19. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is ABCB4, or a variant or a functional fragment thereof.
20. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is ABCB11, or a variant or a functional fragment thereof.
21. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is CDKL5, or a variant or a functional fragment thereof.
22. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is CNTNAP2, or a variant or a functional fragment thereof.
23. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is ZEB2, or a variant or a functional fragment thereof.
24. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is Factor V, or a variant or a functional fragment thereof.
25. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is Factor VIII, or a variant or a functional fragment thereof.
26. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is Factor IX, or a variant or a functional fragment thereof.
27. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is Factor X, or a variant or a functional fragment thereof.
28. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is a blood clotting factor.
29. The expression cassette of clause 28, wherein the blood clotting factor is any one of Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12, or a variant or a functional fragment thereof.
30. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is a DNA binding protein.

31. The expression cassette of clause 30, wherein the DNA binding protein is a transcriptional modulator of an endogenous gene.
32. The expression cassette of clause 31, wherein the transcriptional modulator is a transcriptional activator.
33. The expression cassette of clause 32, wherein the endogenous gene is under-expressed in vivo.
34. The expression cassette of any one of clauses 30-33, wherein the endogenous gene is associated with a liver disease or condition.
35. The expression cassette of any one of clauses 30-33, wherein the endogenous gene is associated with progressive familial intrahepatic cholestasis (PFIC) type 1, type 2, or type 3.
36. The expression cassette of any one of clauses 30-33, wherein the endogenous gene is associated with autism spectrum disorder.
37. The expression cassette of any one of clauses 30-33, wherein the endogenous gene is associated with hemophilia.
38. The expression cassette of any one of clauses 30-33, wherein the endogenous gene is associated with Wilson's disease.
39. The expression cassette of any one of clauses 30-33, wherein the endogenous gene is associated with Menkes disease or occipital horn syndrome.
40. The expression cassette of any one of clauses 30-33, wherein the endogenous gene is CDKL5.
41. The expression cassette of clause 35, wherein the endogenous gene is ABCB11, which is associated with progressive familial intrahepatic cholestasis is type 2 (PFIC2).
42. The expression cassette of clause 35, wherein the endogenous gene is ATP8B1, which is associated with progressive familial intrahepatic cholestasis is type 1 (PFIC1).
43. The expression cassette of clause 35, wherein the endogenous gene is ABCB4, which is associated with progressive familial intrahepatic cholestasis is type 3 (PFIC3).
44. The expression cassette of clause 36, wherein the endogenous gene is CNTNAP2, which is associated with autism spectrum disorder.
45. The expression cassette of clause 37, wherein the endogenous gene is Factor VIII or a variant thereof, which is associated with hemophilia A.
46. The expression cassette of clause 37, wherein the endogenous gene is Factor IX or a variant thereof, which is associated with hemophilia B.

47. The expression cassette of clause 37, wherein the endogenous gene is any one of Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12.
48. The expression cassette of clause 38, wherein the endogenous gene is ATP7B or a variant thereof, which is associated with Wilson's disease.
49. The expression cassette of clause 39, wherein the endogenous gene is ATP7A or a variant thereof, Menkes disease or occipital horn syndrome.
50. The expression cassette of any one of clauses 1-14, wherein the therapeutic transgene is a gene editing protein.
51. The expression cassette of clause 50, wherein the gene editing protein is a Cas protein.
52. The expression cassette of clause 51, further comprising a guide RNA that targets the Cas protein to a genomic locus comprising an endogenous gene.
53. The expression cassette of clause 52, wherein the endogenous gene comprises a mutation or is expressed abnormally.
54. The expression cassette of clause 53, wherein the mutation results in an under-expression of the endogenous gene.
55. The expression cassette of any one of clauses 51-54, wherein the endogenous gene is ATP7A, ATP7B, ATP8B1, ABCB4, ABCB11, CDKL5, CNTNAP2, ZEB2, or Factor VIII.
56. The expression cassette of any one of clauses 51-54, wherein the endogenous gene is Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12.
57. The expression cassette of any one of clauses 1-56, wherein the regulatory element results in increased expression of the therapeutic transgene or the endogenous gene by at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 15 fold, at least 20 fold, at least 25 fold, at least 50 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, or at least 1000 fold as compared to an expression cassette without the regulatory element.
58. The expression cassette of clause 57, wherein the increased expression is determined by amount of RNA transcripts from the therapeutic transgene or the endogenous using a PCR assay.

59. The expression cassette of clause 57, wherein the increased expression is determined by level of protein synthesized from the therapeutic transgene or the endogenous gene using an immunoassay.
60. The expression cassette of any one of clauses 30-56, wherein the regulatory element results in increased expression of the endogenous gene by at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 15 fold, at least 20 fold, at least 25 fold, at least 50 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, or at least 1000 fold as compared to an expression cassette without the regulatory element.
61. The expression cassette of any one of clauses 30-56, wherein the regulatory element results in increased expression of the endogenous gene at a level comparable to expression of the endogenous gene in a healthy subject.
62. The expression cassette of any one of clauses 60-61, wherein the increased expression is determined by amount of RNA transcripts from the endogenous gene using a PCR assay.
63. The expression cassette of any one of clauses 60-61, wherein the increased expression is determined by level of protein synthesized from the endogenous gene using an immunoassay.
64. A method of treating Wilson's disease, comprising administering an expression cassette according to clause 1-16, 30-34, 38, 48, 50-55, and 57-63.
65. A method of treating a genetic defect in ATP7B, comprising administering the expression cassette according to any one of clauses 1-16, 30-34, 38, 48, 50-55, and 57-63.
66. A method of treating a blood clotting disorder, comprising administering the expression cassette according to any one of clauses 1-15, 24-34, 37, 45-47, and 50-63.
67. A method of treating a haploinsufficiency or a genetic defect, comprising administering the expression cassette according to any one of clauses 1-66.
68. The method of clause 67, wherein the therapeutic transgene or the endogenous gene is ATP7A, ATP7B, ATP8B1, ABCB4, ABCB11, CDKL5, CNTNAP2, ZEB2, or Factor VIII, or a variant thereof.
69. The method of clause 67, wherein the therapeutic transgene or the endogenous gene is Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12, or a variant thereof.

70. The method of clause 67 or 68, wherein the transgene is ATP7B, or a variant or a functional fragment thereof.
71. The method of clause 67 or 68, wherein the transgene is a transcriptional modulator that modulates expression of an endogenous gene.
72. The method of clause 71, wherein the endogenous gene is ATP7A, ATP7B, ATP8B1, ABCB4, ABCB11, CDKL5, CNTNAP2, ZEB2, or Factor VIII, or a variant thereof.
73. A method of producing a recombinant protein or a biologic, comprising introducing the expression cassette according to any one of clauses 1-63 into a cell.
74. An AAV expression cassette comprising a human-derived regulatory element of no more than 120bp operably linked to a transgene of at least 3kb, wherein the regulatory element results in increased transgene expression by at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 15 fold, at least 20 fold, at least 25 fold, at least 50 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, or at least 1000 fold as compared to expression of the transgene when operably linked to a CMV promoter.
75. An AAV expression cassette comprising a relatively short human-derived regulatory element operably linked to a transgene of at least 3kb, wherein the relatively short regulatory element results in increased transgene expression by at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 15 fold, at least 20 fold, at least 25 fold, at least 50 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, or at least 1000 fold as compared to a comparable expression cassette without the regulatory element.
76. The AAV expression cassette of clause 74 or 75, wherein the regulatory element comprises (i) SEQ ID NO: 1-2, 13-17, and 22-41; (ii) a combination thereof; or (iii) or sequence having at least 80%, 85%, 90%, or 95% sequence identity to any one of (i) and (ii).
77. The AAV expression cassette of any one of clauses 74-76, wherein the increased transgene expression is at least 50 fold as compared to expression of the transgene when operably linked to a CMV promoter.

78. The AAV expression cassette of clause 77, wherein the increased transgene expression is at least 100 fold.
79. The AAV expression cassette of any one of clauses 74-78, wherein the regulatory element exhibits a size-normalized expression activity that is at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 15 fold, at least 20 fold, at least 25 fold, at least 50 fold, or at least 100 fold as compared to size-normalized expression activity of a CMV promoter operably linked to the transgene.
80. The AAV expression cassette of any one of clauses 74-79, wherein the increased transgene expression occurs in at least 2 cell types.
81. The AAV expression cassette of clause 80, wherein the at least 2 cell types are selected from the group consisting of: kidney cells, epithelial cells, neurons, and liver cells.
82. The AAV expression cassette of any one of clauses 74-81, wherein the regulatory element comprises any one or more of SEQ ID NOs: 22-41, and wherein no other promoter sequences are present in the expression cassette.
83. The AAV expression cassette of any one of clauses 74-82, wherein the regulatory element comprises SEQ ID NO: 1 or SEQ ID NO: 2.
84. The AAV expression cassette of clause 83, wherein the regulatory element is located downstream of a promoter.
85. The AAV expression cassette of any one of clauses 74-84, wherein the regulatory element is located upstream of the transgene.
86. The AAV expression cassette of any one of clauses 74-85, wherein the transgene is ATP7A; ATP7B; ATP8B1; ABCB4; ABCB11; CDKL5; CNTNAP2; ZEB2; Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12; or a variant or a functional fragment thereof.
87. The AAV expression cassette of clause 86, wherein the transgene is ATP7B, or a variant or a functional fragment thereof.
88. The AAV expression cassette of clause 86, wherein the transgene is FVIII, or a variant or a functional fragment thereof.
89. The AAV expression cassette of any one of clauses 74-85, wherein the transgene is a gene editing protein.
90. The AAV expression cassette of clause 89, wherein the gene editing protein is Cas.
91. The AAV expression cassette of any one of clauses 74-85, wherein the transgene is a DNA binding protein.

92. The AAV expression cassette of clause 91, wherein the DNA binding protein is a transcriptional activator that increases expression of an endogenous gene.
93. The AAV expression cassette of clause 91, wherein the DNA binding protein is a transcriptional repressor that decreases expression of an endogenous gene.
94. The AAV expression cassette of clause 92, wherein the transcriptional activator increases expression of ATP7A; ATP7B; ATP8B1; ABCB4; ABCB11; CDKL5; CNTNAP2; ZEB2; or Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12, or a variant thereof.
95. The AAV expression cassette of clause 94, wherein the transcriptional activator increases expression of an endogenous ATP7A.
96. The AAV expression cassette of clause 94, wherein the transcriptional activator increases expression of an endogenous ATP7B.
97. The AAV expression cassette of clause 94, wherein the transcriptional activator increases expression of an endogenous ATP8B1.
98. The AAV expression cassette of clause 94, wherein the transcriptional activator increases expression of an endogenous ABCB4.
99. The AAV expression cassette of clause 94, wherein the transcriptional activator increases expression of an endogenous ABCB11.
100. The AAV expression cassette of clause 94, wherein the transcriptional activator increases expression of an endogenous FVIII.
101. The AAV expression cassette of any one of clauses 74-100, wherein the AAV is selected from the group consisting of: AAV1, AAV2, AAV3, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV-DJ, and scAAV.
102. The AAV expression cassette of clause 101, wherein the AAV is AAV8.
103. A method of producing a recombinant protein, the method comprising operably linking a sequence encoding the protein with one or more of (i) SEQ ID NO: 1-2, 13-17, and 22-41; (ii) a combination thereof; or (iii) sequences having at least 80%, 85%, 90%, or 95% sequence identity to any one of (i) and (ii).
104. The method of clause 103, wherein the sequence encoding the protein is more than 1kb, 1.5kb, 2kb, 2.5kb, 3kb, 3.5kb, 4kb, 4.5kb, 5kb, 5.5kb, 6kb, 6.5kb, 7kb, or 7.5kb.
105. A method of treating a liver disease or condition, comprising administering a gene therapy comprising a therapeutic transgene operably linked to one or more regulatory elements selected from (i) SEQ ID NO: 1-2, 13-17, and 22-41; (ii) a combination

thereof; or (iii) sequences having at least 80%, 85%, 90%, or 95% sequence identity to any one of (i) and (ii).

106. The method of clause 105, wherein the liver disease or condition is Wilson's disease.

107. The method of clause 105, wherein the liver disease or condition is a blood clotting disorder.

108. The method of clause 105, wherein the transgene is ATP7A or ATP7B, or a variant or a functional fragment thereof.

109. The method of clause 105, wherein the transgene is Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or a variant or a functional fragment thereof.

110. The method of clause 109, wherein the transgene is Factor 8, or a variant or a functional fragment thereof.

111. The method of any one of clauses 105-110, wherein the regulatory elements result in increased transgene expression in at least 2 cell types.

112. The method of any one of clauses 150-110, wherein the regulatory elements result in increased transgene expression in at least 3 cell types.

113. The method of any one of clauses 105-112, wherein the regulatory elements result in increased transgene expression in hepatocytes.

114. The method of any one of clauses 105-113, wherein the regulatory elements result in increased transgene expression at a level that is at least 2 fold as compared to expression of the transgene when operably linked to a CMV promoter.

115. The method of any one of clauses 105-114, wherein the gene therapy utilizes AAV.

116. The method of clause 115, wherein the AAV is AAV8.

117. The method of any one of clauses 105-114, wherein the gene therapy utilizes an adenovirus.

118. The method of any one of clauses 105-114, wherein the gene therapy utilizes a lentivirus.

119. An expression vector comprising: a human-derived regulatory element having less than or equal to100bp operably linked to a transgene, wherein the regulatory element increases global expression of the transgene by at least two fold as compared to a second expression vector without the regulatory element.

120. The expression vector of clause 119, wherein the second expression vector comprises the transgene operably linked to a CMV promoter.

121. An expression vector comprising a human-derived regulatory element having less than or equal to 100bp operably linked to a transgene, whereby expression of the transgene is higher than that of the same transgene expressed by a CMV promoter, super core promoter, TTR promoter, Proto 1 promoter, UCL-HLP promoter, or CMVe promoter.
122. The expression vector of clause 121, wherein the expression of the transgene is higher than that of the same transgene expressed by a UCL-HLP promoter.
123. An expression vector comprising a human-derived regulatory element operably linked to a transgene, wherein a protein encoded by the transgene has (i) a concentration >1.0 IU/mL as measured by an ELISA assay configured to detect the transgene; or (ii) > 25% activity as measured by a Coatest assay.
124. A vector comprising a human-derived regulatory element having a sequence less than or equal to 100bp in size operably linked to a transgene not found in context with the regulatory element in a cell or in vivo.
125. The vector of any one of clauses 119-124, wherein the regulatory element is an intronic sequence.
126. The vector of clause 125, wherein the regulatory element is SEQ ID NO: 1 or SEQ ID NO: 2, or a sequence having at least 80%, 85%, 90%, or 95% homology thereto.
127. The expression vector or cassette of any one of clauses 1-63, 74-102, and 119-126, wherein use of a luciferase as the transgene results in global expression that is greater than $1x10^8$ photons/sec as measured in whole mice.
128. The expression vector or cassette of clause 127, wherein the activity or transgene expression corresponds to a dose of 16 µg of expression vector per mouse.
129. The expression vector or cassette of clause 127, wherein the activity or transgene expression corresponds to a dose of 12 µg of expression vector per mouse.
130. The expression vector or cassette of any one of clauses 1-63, 74-102, and 119-129, wherein the endogenous version of the transgene is not linked to the regulatory element in vivo.
131. The expression vector or cassette of clause 127, wherein the global expression of the transgene is at a level greater than expression of the transgene using a vector or cassette with a regulatory element selected from the group consisting of: a CMV promoter, a CMVe promoter, a super core promoter, TTR promoter, Proto 1 promoter, and a UCL-HLP promoter.

132. The expression vector or cassette of any one of clauses 1-63, 74-102, and 119-131, wherein expression is detectable in at least 3, 4, 5, 6, or 7 different cell types in mouse in vivo.

133. The expression vector or cassette of clause 132, wherein the different cell types are selected from the group consisting of: alveolar cells, cardiomyocytes, epithelial cells, hepatocytes, intestinal cells, myocytes, neurons, and renal cells.

134. The expression vector or cassette of any one of clauses 119-133, wherein the regulatory element is an enhancer.

135. The expression vector or cassette of clause 134, further comprising a promoter.

136. The expression vector or cassette of clause 135, wherein the promoter is a CMV promoter, CMV, promoter, super core promoter, TTR promoter, Proto 1 promoter, UCL-HLP promoter, an AAT promoter, a KAR promoter, a EF1α promoter, EFS promoter, or CMVe enhancer/CMV promoter combination.

137. The expression vector of any one of clauses 119-136, further comprising one or more post-transcriptional modification sites.

138. The expression vector of any one of clauses 119-137, wherein the transgene is Cas9.

139. The expression vector of clause 138, wherein the transgene is saCas9.

140. An expression vector comprising a Factor VIII transgene operably linked to a regulatory sequence that is able to drive expression of the Factor VIII to a concentration >1.0 IU/mL as measured by an ELISA assay configured to detect Factor VIII in a cell or in vivo.

141. The expression vector of clause 140, wherein the regulatory sequence is relatively short.

142. The expression vector of clause 140 or 141, wherein the regulatory sequence comprises one or more of SEQ ID NO: 1-2, 13-17, and 22-41, or a combination thereof; or sequences having at least 80%, 85%, 90%, or 95% sequence identity thereto.

143. A viral particle comprising the expression vector of any one of clauses 119-142.

144. The viral particle of clause 143, wherein the viral particle is an AAV.

145. The viral particle of clause 144, wherein the AAV is selected from the group consisting of: AAV1, AAV2, AAV3, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV-DJ, and scAAV.

146. The viral particle of clause 143, wherein the viral particle is a lentivirus.

147. The viral particle of clause 143, wherein the virus particle is an adenovirus.

148. The expression vector of clause 119-147, wherein the transgene is a therapeutic transgene.

149. The expression vector of clause 148, wherein the therapeutic transgene is Factor VIII, Cas9, a DNA binding protein, hormone, growth or differentiation factor, insulin, growth hormone, VEGF, neurotrophic factor, fibroblast epithelial factor; cytokine, interleukin, lymphokine, tumor necrosis factor, antibody, immunoglobulin, interferon, chimeric T cell receptor; lipoprotein receptor, cystic fibrosis transmembrane regulator, a gene associated with mucopolysaccharidosis type I, II, III, or IV, beta globin or lipoprotein lipase.

150. The expression vector of clause 148, wherein the therapeutic transgene is ATP7A, ATP7B, ATP8B1, ABCB4, ABCB11, or a variant or functional fragment thereof.

151. The expression vector of clause 148, wherein the therapeutic transgene is CDKL5, CNTNAP2, ZEB2, or a variant or functional fragment thereof.

152. A method for delivering a transgene to a plurality of different tissues in an animal comprising administering to said animal an expression vector of any one of clauses 119-151.

153. A method for production of proteins, antibodies, or other biologics, comprising contacting a cell with an expression vector of any one of clauses 119-151.

154. The method of clause 153, wherein the cell is a CHO cell or a HEK293T cell.

155. A method for producing a transgenic animal or plant comprising administering to an animal or plant an expression vector of any one of clauses 119-151.

156. The expression vector of any one of clauses 119-151, wherein the regulatory element is 40-50bp.

157. The expression vector of any one of clauses 119-151, wherein the regulatory element is 50-60bp.

158. An expression vector comprising a human-derived regulatory element operably linked to a transgene, wherein a protein encoded by the transgene has (i) a concentration >0.1 IU/mL as measured by an ELISA assay configured to detect the transgene; or (ii) > 10% activity as measured by a Coatest assay.

159. An expression vector comprising a human-derived regulatory element having less than or equal to 120bp operably linked to a transgene, whereby expression of the transgene is higher than that of the same transgene expressed by a UCL-HLP promoter.

160. The expression vector of any one of clauses 158-159, wherein the regulatory element is a promoter.

161. The expression vector of any one of clauses 158-160, wherein the therapeutic transgene is a fusion protein comprising a DNA binding domain and a transcription regulatory domain.

162. An expression cassette comprising a relatively short regulatory element (RE) operably linked to a therapeutic transgene.

163. The expression cassette of clause 162, wherein the relative short RE comprises one or more of (i) SEQ ID NOs: 1-2, 13-17, and 22-41; or (ii) sequences having at least 80%, 85%, 90%, or 95% sequence identity to any one of (i).

164. The method of any one of clauses 64-72 and 105-118, wherein the administering is systemic.

165. The method of clause 164, wherein the administering comprises intravenous injection or infusion in a subject.

166. The method of clause 165, wherein the subject is a human.

167. The method of clause 165, wherein the subject is an animal.

CLAIMS

WHAT IS CLAIMED IS:

1. An expression cassette comprising a regulatory element operably linked to a therapeutic transgene, wherein the regulatory element comprises one or more of (i) SEQ ID NOs: 1-2, 13-17, and 22-41; or (ii) sequences having at least 80% sequence identity to any one of (i).

2. The expression cassette of claim 1, wherein the regulatory element is non-naturally occurring.

3. The expression cassette of claim 1, wherein the regulatory element comprises an intronic sequence.

4. The expression cassette of claim 3, wherein the regulatory element is located between a promoter and the therapeutic transgene.

5. The expression cassette of claim 1, wherein the regulatory element comprises a promoter sequence.

6. The expression cassette of claim 5, wherein the regulatory element is the only promoter in the cassette.

7. The expression cassette of claim 1, wherein the regulatory element is no more than 100bp.

8. The expression cassette of claim 1, wherein the regulatory element is no more than 60bp.

9. The expression cassette of claim 1, wherein the regulatory element is no more than 50bp.

10. The expression cassette of claim 1, wherein the expression cassette is part of a rAAV.

11. The expression cassette of claim 1, wherein the rAAV is rAAV8.

12. The expression cassette of any one of claims 1-11, wherein the therapeutic transgene is ATP7B or a variant thereof.

13. The expression cassette of any one of claims 1-11, wherein the therapeutic transgene is Factor VIII or a variant thereof.

14. A method of treating Wilson's disease, comprising administering the expression cassette according to any one of claims 1-11 and wherein the transgene is ATP7B or a variant thereof.

15. A method of treating a genetic defect in ATP7B, comprising administering the expression cassette according to any one of claims 1-11 and wherein the transgene is ATP7B or a variant thereof.

16. A method of treating a blood clotting disorder, comprising administering the expression cassette according to any one of claims 1-11 and wherein the transgene is Factor VIII or a variant thereof.

17. A method of treating a haploinsufficiency or a genetic defect, comprising administering the expression cassette according to any one of claims 1-11.

18. The method of claim 17, wherein the transgene is ATP7A or a variant thereof.

19. The method of claim 17, wherein the transgene is ATP7B or a variant thereof.

20. The method of claim 17, wherein the transgene is ATP8B1 or a variant thereof.

21. The method of claim 17, wherein the transgene is ABCB4 or a variant thereof.

22. The method of claim 17, wherein the transgene is ABCB11 or a variant thereof.

23. The method of claim 17, wherein the transgene is CDKL5 or a variant thereof.

24. The method of claim 17, wherein the transgene is CNTNAP2 or a variant thereof.

25. The method of claim 17, w herein the transgene is ZEB2 or a variant thereof.

26. The method of claim 17, wherein the transgene is Factor V or a variant thereof.

27. The method of claim 17, wherein the transgene is Factor VII or a variant thereof.

28. The method of claim 17, wherein the transgene is Factor VIII or a variant thereof.

29. The method of claim 17, wherein the transgene is Factor IX or a variant thereof.

30. The method of claim 17, wherein the transgene is Factor XI or a variant thereof.

31. A method of treating a haploinsufficiency or a genetic defect, comprising administering the expression cassette according to any one of claims 1-11 and wherein the transgene is a transcriptional modulator that modulates expression of an endogenous gene.

32. The method of claim 31, wherein the transcriptional modulator is a transcriptional activator of the endogenous gene.

33. The method of claim 32, wherein the endogenous gene is ATP7A.

34. The method of claim 32, wherein the endogenous gene is ATP7B.

35. The method of claim 32, wherein the endogenous gene is ATP8B1.

36. The method of claim 32, wherein the endogenous gene is ABCB4.

37. The method of claim 32, wherein the endogenous gene is ABCB11.

38. The method of claim 32, wherein the endogenous gene is CDKL5.

39. The method of claim 32, wherein the endogenous gene is CNTNAP2.

40. The method of claim 32, wherein the endogenous gene is ZEB2.

41. The method of claim 32, wherein the transgene is Factor V.

42. The method of claim 32, wherein the transgene is Factor VII.

43. The method of claim 32, wherein the transgene is Factor VIII.

44. The method of claim 32, wherein the transgene is Factor IX.

45. The method of claim 32, wherein the transgene is Factor XI.

46. An AAV expression cassette comprising a human-derived regulatory element of no more than 120bp operably linked to a transgene of at least 3kb, wherein the regulatory element results in increased transgene expression by at least 2 fold as compared to expression of the transgene when operably linked to a CMV promoter.

47. The AAV expression cassette of claim 46, wherein the regulatory element comprises (i) SEQ ID NO: 1-2, 13-17, and 22-41; (ii) a combination thereof; or (iii) or sequence having at least 80% sequence identity to any one of (i) and (ii).

48. The AAV expression cassette of claim 46, wherein the increased transgene expression is at least 50 fold as compared to expression of the transgene when operably linked to a CMV promoter.

49. The AAV expression cassette of claim 46, wherein the increased transgene expression is at least 100 fold.

50. The AAV expression cassette of claim 46, wherein the regulatory element exhibits a size-normalized expression activity that is at least 1.5 fold as compared to size-normalized expression activity of a CMV promoter operably linked to the transgene.

51. The AAV expression cassette of claim 46, wherein the increased transgene expression occurs in at least 2 different cell types.

52. The AAV expression cassette of claim 51, wherein the at least 2 different cell types are selected from kidney cells, neurons, and liver cells.

53. The AAV expression cassette of claim 46, wherein the regulatory element comprises any one or more of SEQ ID NOs: 22-41, and wherein no other promoter sequences are present in the expression cassette.

54. The AAV expression cassette of claim 46, wherein the regulatory element comprises SEQ ID NO: 1 or SEQ ID NO: 2.

55. The AAV expression cassette of claim 54, wherein the regulatory element is located downstream of a promoter.

56. The AAV expression cassette of claim 46, wherein the transgene is any one of ATP7a; ATP7B; ATP8B1; ABCB4; ABCB11; CDKL5; CNTNAP2; ZEB2; Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12; or a variant or a functional fragment thereof.

57. The AAV expression cassette of claim 46, wherein the transgene is ATP7B or a variant thereof.

58. The AAV expression cassette of claim 46, wherein the transgene is FVIII or a variant thereof.

59. The AAV expression cassette of claim 46, wherein the transgene is a gene editing protein.

60. The AAV expression cassette of claim 59, wherein the gene editing protein is Cas.

61. The AAV expression cassette of claim 46, wherein the transgene is a DNA binding protein.

62. The AAV expression cassette of claim 61, wherein the DNA binding protein is a transcriptional activator that increases expression of an endogenous gene.

63. The AAV expression cassette of claim 61, wherein the transgene is a transcriptional repressor that decreases expression of an endogenous gene.

64. The AAV expression cassette of claim 62, wherein the transcriptional activator increases expression of any one of the following endogenous genes: ATP7A; ATP7B; ATP8B1; ABCB4; ABCB11; CDKL5; CNTNAP2; ZEB2; and Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12.

65. The AAV expression cassette of claim 62, wherein the transcriptional activator increases expression of an endogenous ATP7A.

66. The AAV expression cassette of claim 62, wherein the transcriptional activator increases expression of an endogenous ATP7B.

67. The AAV expression cassette of claim 62, wherein the transcriptional activator increases expression of an endogenous ATP8B1.

68. The AAV expression cassette of claim 62, wherein the transcriptional activator increases expression of an endogenous ABCB4.

69. The AAV expression cassette of claim 62, wherein the transcriptional activator increases expression of an endogenous ABCB11.

70. The AAV expression cassette of claim 62, wherein the transcriptional activator increases expression of an endogenous FVIII.

71. The AAV expression cassette of any one of claims 46-70, wherein the AAV is selected from the group consisting of: AAV1, AAV2, AAV3, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV-DJ, and scAAV.

72. The AAV expression cassette of claim 71, wherein the AAV is AAV8.

73. A method of producing a recombinant protein, the method comprising operably linking a sequence encoding the protein with one or more of (i) SEQ ID NO: 1-2, 13-17, and 22-41; (ii) a combination thereof; or (iii) sequences having at least 80% sequence identity to any one of (i) and (ii).

74. A method of treating a liver disease or condition, comprising administering a gene therapy comprising a therapeutic transgene operably linked to one or more regulatory elements selected from (i) SEQ ID NO: 1-2, 13-17, and 22-41; (ii) a combination thereof; or (iii) sequences having at least 80% sequence identity to any one of (i) and (ii).

75. The method of claim 74, wherein the liver disease or condition is Wilson's disease.

76. The method of claim 74, wherein the liver disease or condition is a blood clotting disorder.

77. The method of claim 75, wherein the transgene is ATP7A or ATP7B, or a variant or a functional fragment thereof.

78. The method of claim 76, wherein the transgene is Factor 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or a variant or a functional fragment thereof.

79. The method of claim 78, wherein the transgene is Factor 8, or a variant or a functional fragment thereof.

80. The method of claim 74, wherein the regulatory elements result in increased transgene expression in at least 2 cell types.

81. The method of claim 74, wherein the regulatory elements result in increased transgene expression in at least 3 cell types.

82. The method of claim 74, wherein the regulatory elements result in increased transgene expression in hepatocytes.

83. The method of any one of claims 74-82, wherein the regulatory elements result in increased transgene expression at a level that is at least 2 fold as compared to expression of the transgene when operably linked to a CMV promoter.

84. The method of claim 74, wherein the gene therapy utilizes AAV.

85. The method of claim 84, wherein the AAV is AAV8.

86. An expression vector comprising: a human-derived regulatory element having less than or equal to100bp operably linked to a transgene, wherein the regulatory element increases global expression of the transgene by at least two fold as compared to a second expression vector without the regulatory element.

87. The expression vector of claim 86, wherein the second expression vector comprises the transgene operably linked to a CMV promoter.

88. An expression vector comprising a human-derived regulatory element having less than or equal to 100bp operably linked to a transgene, whereby expression of the transgene is higher than that of the same transgene expressed by a CMV promoter, super core promoter, TTR promoter, Proto 1 promoter, UCL-HLP promoter, or CMVe promoter.

89. The expression vector of claim 88, wherein the expression of the transgene is higher than that of the same transgene expressed by a UCL-HLP promoter.

90. An expression vector comprising a human-derived regulatory element operably linked to a transgene, wherein a protein encoded by the transgene has (i) a concentration >1.0 IU/mL as measured by an ELISA assay configured to detect the transgene; or (ii) > 25% activity as measured by a Coatest assay.

91. A vector comprising a human-derived regulatory element having a sequence less than or equal to 100bp in size operably linked to a transgene not found in context with the regulatory element in a cell or in vivo.

92. The vector of claim 86, 88, 90, or 91, wherein the regulatory element is an intronic sequence.

93. The vector of claim 86, 88, 90, or 91, wherein the regulatory element is SEQ ID NO: 1 or SEQ ID NO: 2, or a sequence having at least 80% homology thereto.

94. The expression vector of claim 86, 88, or 90, wherein use of a luciferase as the transgene results in global expression that is greater than $1x10^8$ photons/sec as measured in whole mice.

95. The expression vector of claim 90, wherein the activity or transgene expression corresponds to a dose of 16 µg of expression vector per mouse.

96. The expression vector of claim 94, wherein the activity or transgene expression corresponds to a dose of 12 μg of expression vector per mouse.

97. The expression vector of claim 86, 88, or 90, wherein the endogenous version of the transgene is not linked to the regulatory element in vivo.

98. The expression vector of claim 86, wherein the global expression of the transgene is at a level greater than expression of the transgene using a vector with a regulatory element selected from the group consisting of: a CMV promoter, a CMVe promoter, a super core promoter, TTR promoter, Proto 1 promoter, and a UCL-HLP promoter.

99. The expression vector of claim 86, 88, 90, or 91, wherein expression is detectable in at least 3, 4, 5, 6, or 7 different cell types in mouse in vivo.

100. The expression vector of claim 88, wherein the different cell types are selected from the group consisting of: alveolar cells, cardiomyocytes, epithelial cells, hepatocytes, intestinal cells, myocytes, neurons, and renal cells.

101. The expression vector of claim 86, 88, 90, or 91, wherein the regulatory element is an enhancer.

102. The expression vector of claim 101, further comprising a promoter.

103. The expression vector of claim 102, wherein the promoter is a CMV promoter, CMV, promoter, super core promoter, TTR promoter, Proto 1 promoter, UCL-HLP promoter, an AAT promoter, a KAR promoter, a EF1α promoter, EFS promoter, or CMVe enhancer/CMV promoter combination.

104. The expression vector of claim 101, further comprising one or more post-transcriptional modification sites.

105. The expression vector of claim 86, 88, 90, or 91, wherein the transgene is Cas9.

106. The expression vector of claim 105, wherein the transgene is saCas9.

107. An expression vector comprising a Factor VIII transgene operably linked to a regulatory sequence that is able to drive expression of the Factor VIII to a concentration >1.0 IU/mL as measured by an ELISA assay configured to detect Factor VIII in a cell or in vivo.

108. A viral particle comprising the expression vector of any one of claims 86-107.

109. The viral particle of claim 108, wherein the viral particle is an AAV.

110. The viral particle of claim 109, wherein the AAV is selected from the group consisting of: AAV1, AAV2, AAV3, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV-DJ, and scAAV.

111. The viral particle of claim 108, wherein the viral particle is a lentivirus.

112. The viral particle of claim 108, wherein the virus particle is an adenovirus.

113. The expression vector of claim 86, 88, 90, or 91, wherein the transgene is a therapeutic transgene.

114. The expression vector of claim 113, wherein the therapeutic transgene is Factor VIII, Cas9, a DNA binding protein, hormone, growth or differentiation factor, insulin, growth hormone, VEGF, neurotrophic factor, fibroblast epithelial factor; cytokine, interleukin, lymphokine, tumor necrosis factor, antibody, immunoglobulin, interferon, chimeric T cell receptor; lipoprotein receptor, cystic fibrosis transmembrane regulator, a gene associated with mucopolysaccharidosis type I, II, III, or IV, beta globin or lipoprotein lipase.

115. The expression vector of claim 113, wherein the therapeutic transgene is ATP7A, ATP7B, ATP8B1, ABCB4, ABCB11, or a variant or fragment thereof.

116. The expression vector of claim 113, wherein the therapeutic transgene is CDKL5, CNTNAP2, ZEB2, or a variant or fragment thereof.

117. A method for delivering a transgene to a plurality of different tissues in an animal comprising administering to said animal an expression vector of any one of claims 86-116.

118. A method for production of proteins, antibodies, or other biologics, comprising contacting a cell with an expression vector of any one of claims 86-116.

119. The method of claim 118, wherein the cell is a CHO cell or a HEK293T cell.

120. A method for producing a transgenic animal or plant comprising administering to an animal or plant an expression vector of any one of claims 86-116.

121. The expression vector of claim 86, 88, 90, or 91, wherein the regulatory element is 40-50bp.

122. The expression vector of claim 86, 88, 90, or 91, wherein the regulatory element is 50-60bp.

123. An expression vector comprising a human-derived regulatory element operably linked to a transgene, wherein a protein encoded by the transgene has (i) a concentration >0.1 IU/mL as measured by an ELISA assay configured to detect the transgene; or (ii) > 10% activity as measured by a Coatest assay.

124. An expression vector comprising a human-derived regulatory element having less than or equal to 120bp operably linked to a transgene, whereby expression of the transgene is higher than that of the same transgene expressed by a UCL-HLP promoter.

125. The expression vector of claim 123 or 124, wherein the regulatory element is a promoter.

126. The expression vector of claim 123 or 124, wherein the therapeutic transgene is a fusion protein comprising a DNA binding domain and a transcription regulatory domain.

FIG. 1A

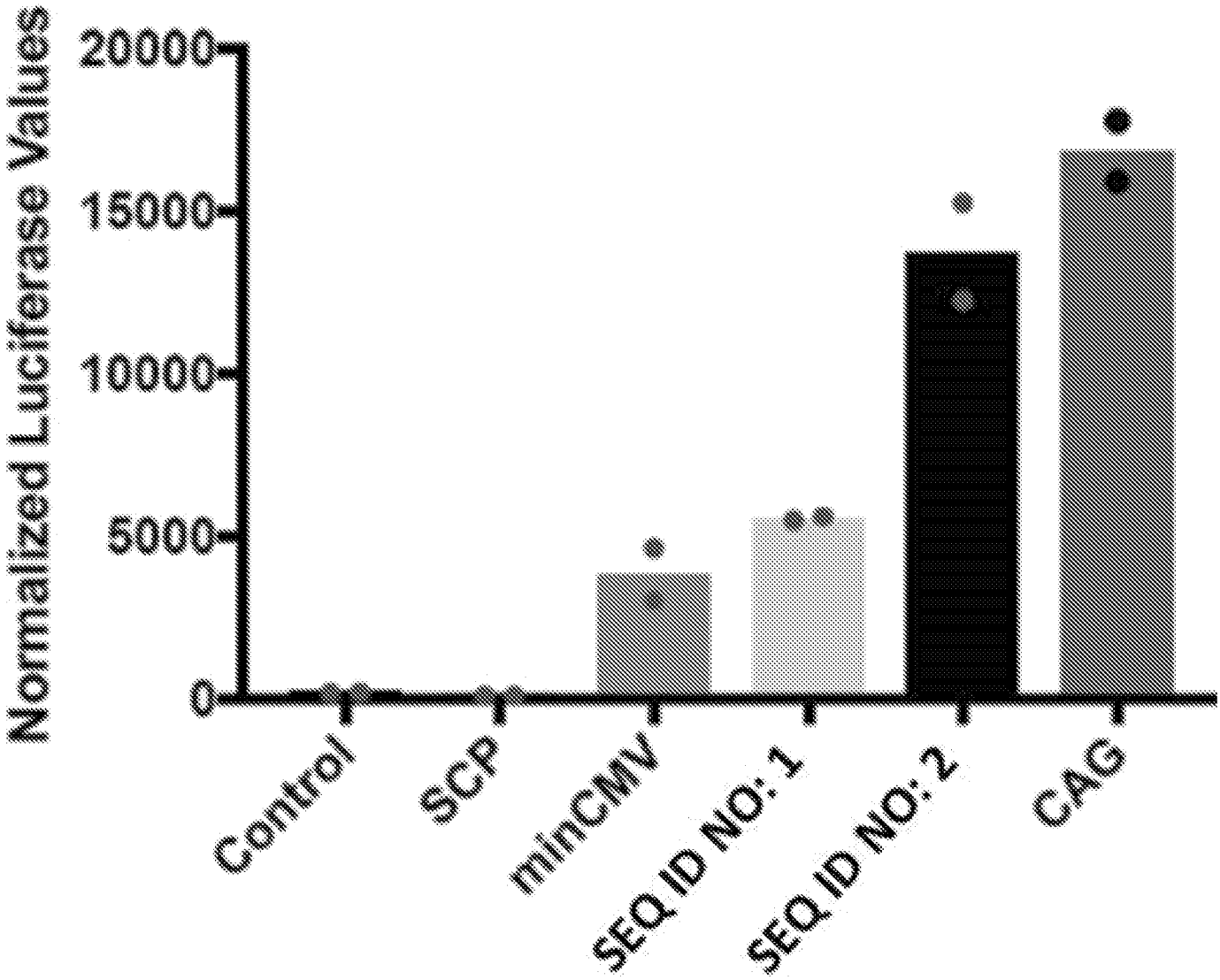

Normalized Luciferase Values
20000
15000
10000
5000
0
Control
SCP
minCMV
SEQ ID NO: 1
SEQ ID NO: 2
CAG

FIG. 1B

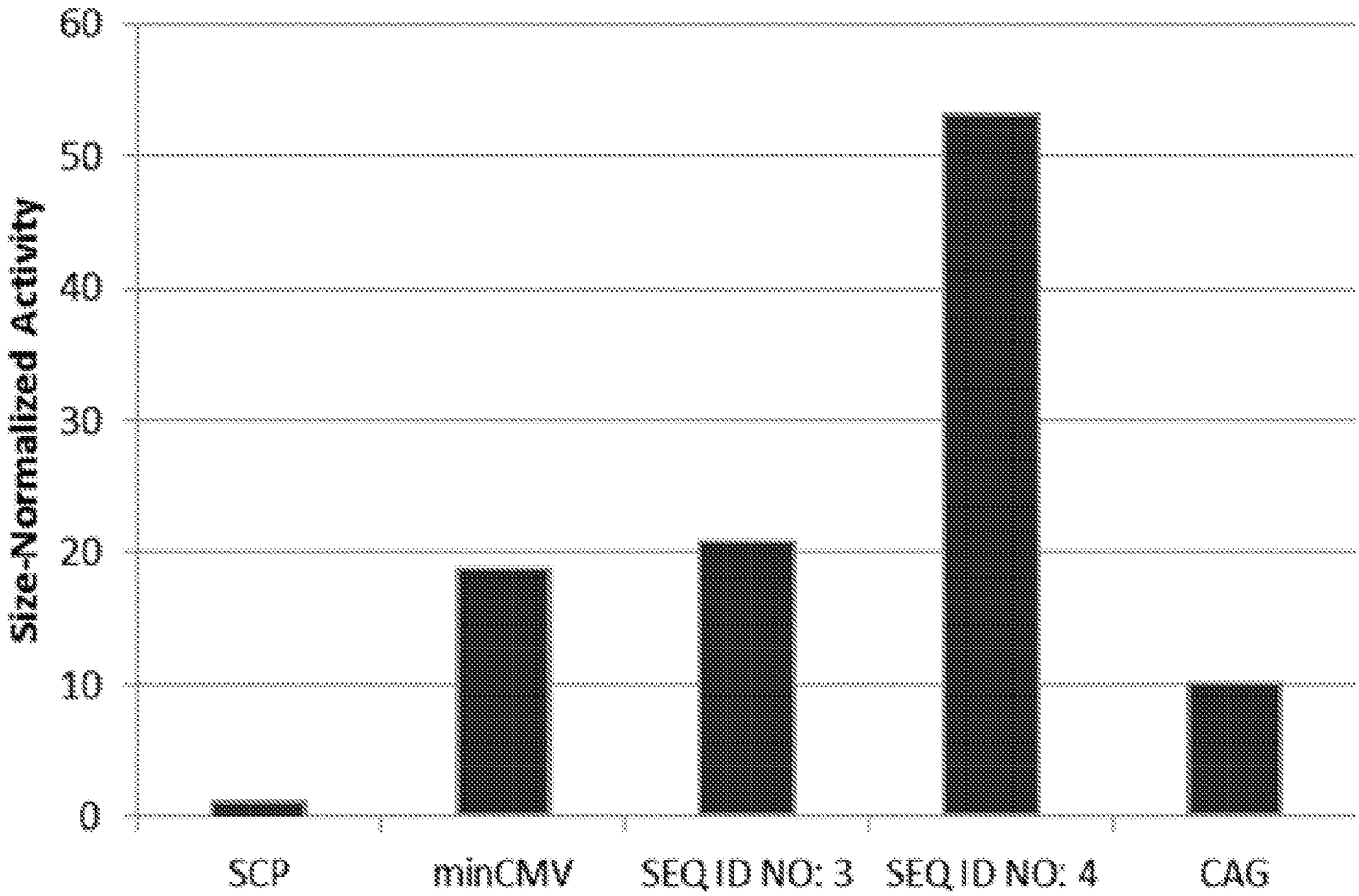

Size-Normalized Activity
60
50
40
30
20
10
0
SCP
minCMV
SEQ ID NO: 3
SEQ ID NO: 4
CAG

FIG. 1C

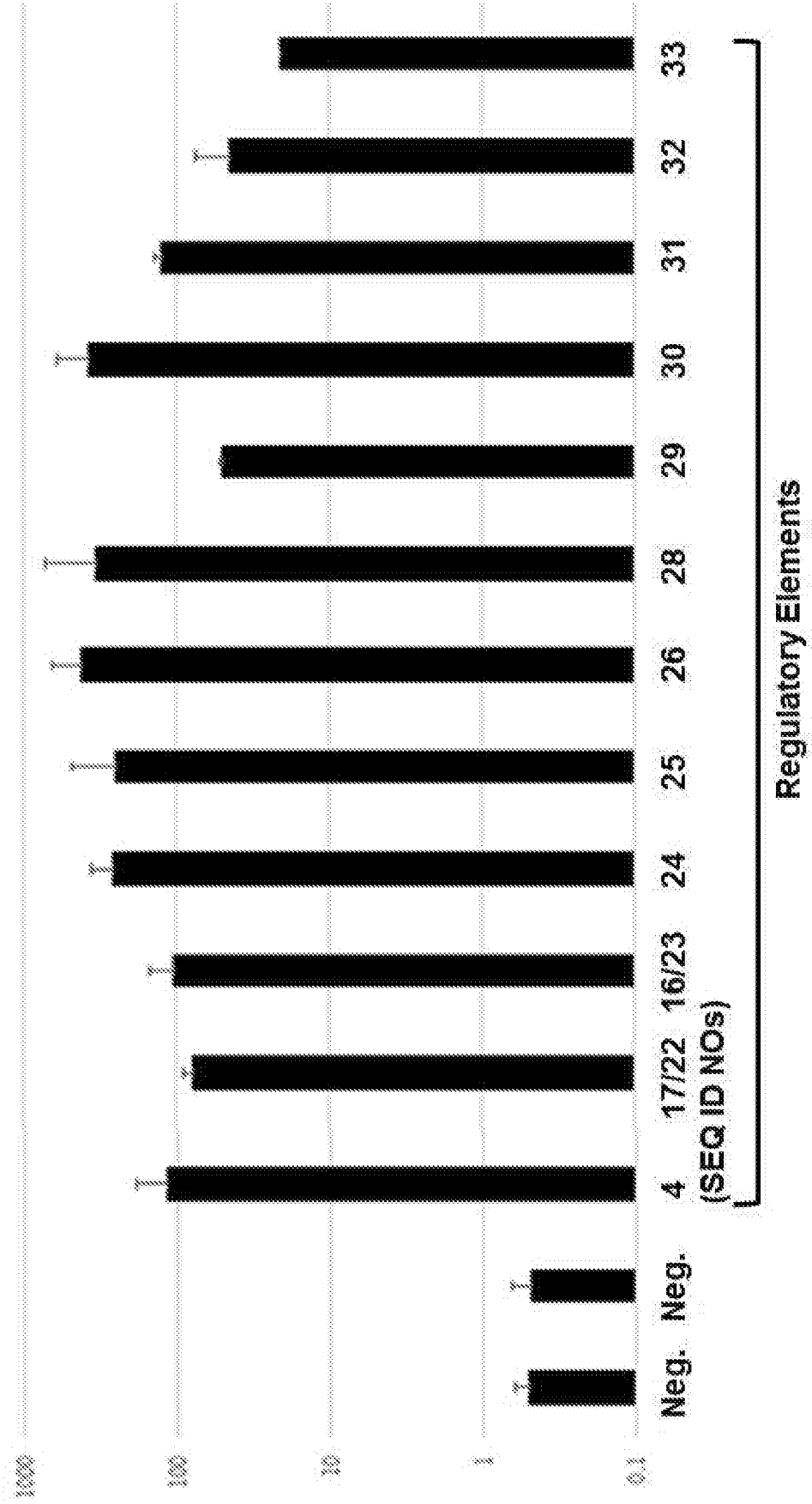

1000
100
10
1
0.1
Neg.
Neg.
4
17/22
16/23
24
25
26
28
29
30
31
32
33
(SEQ ID NOs)
Regulatory Elements

FIG. 1D

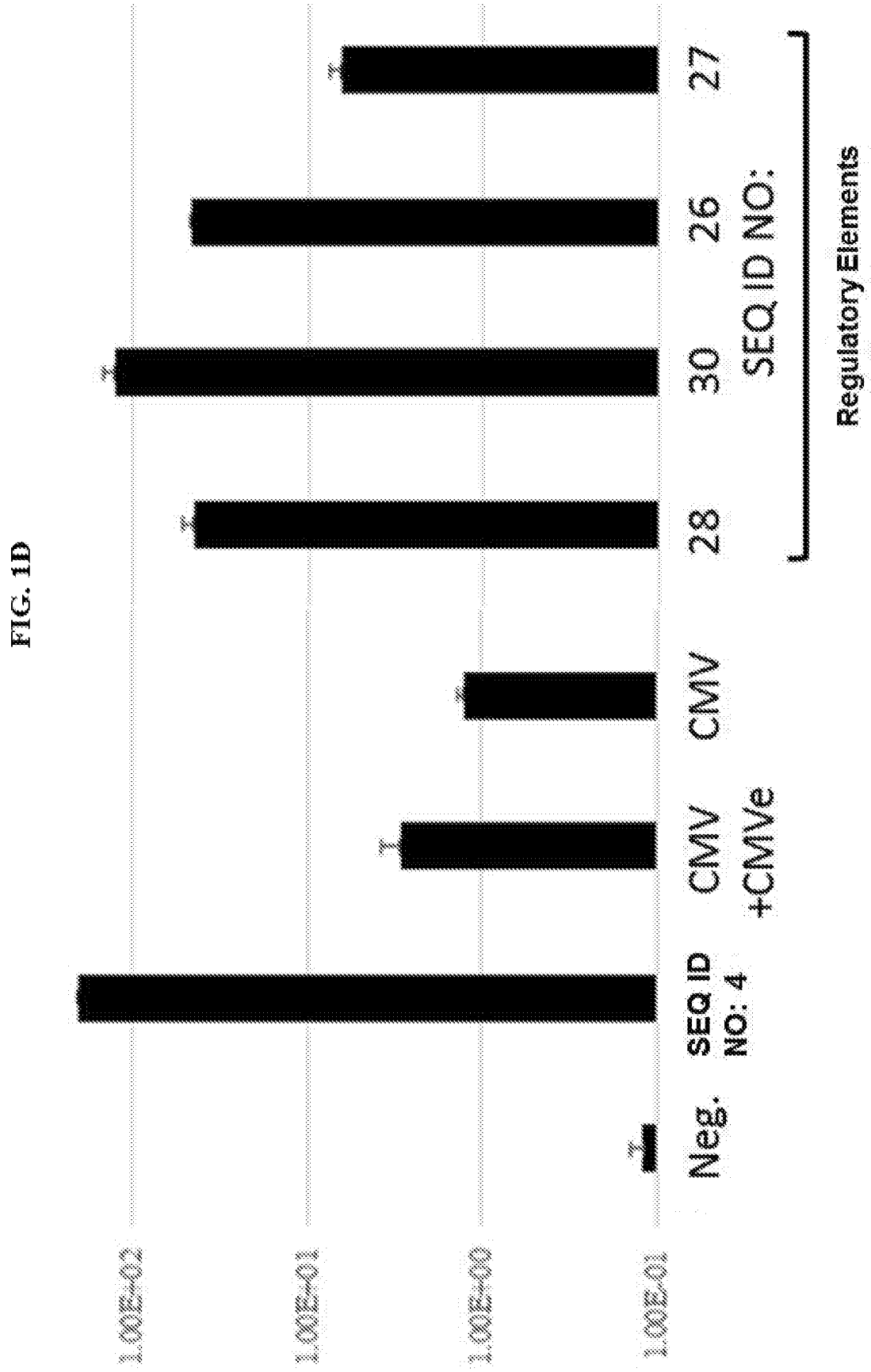

1.00E+02
1.00E+01
1.00E+00
1.00E-01
Neg.
SEQ ID NO: 4
CMV +CMVe
CMV
28
30
26
27
SEQ ID NO:
Regulatory Elements

FIG. 1E

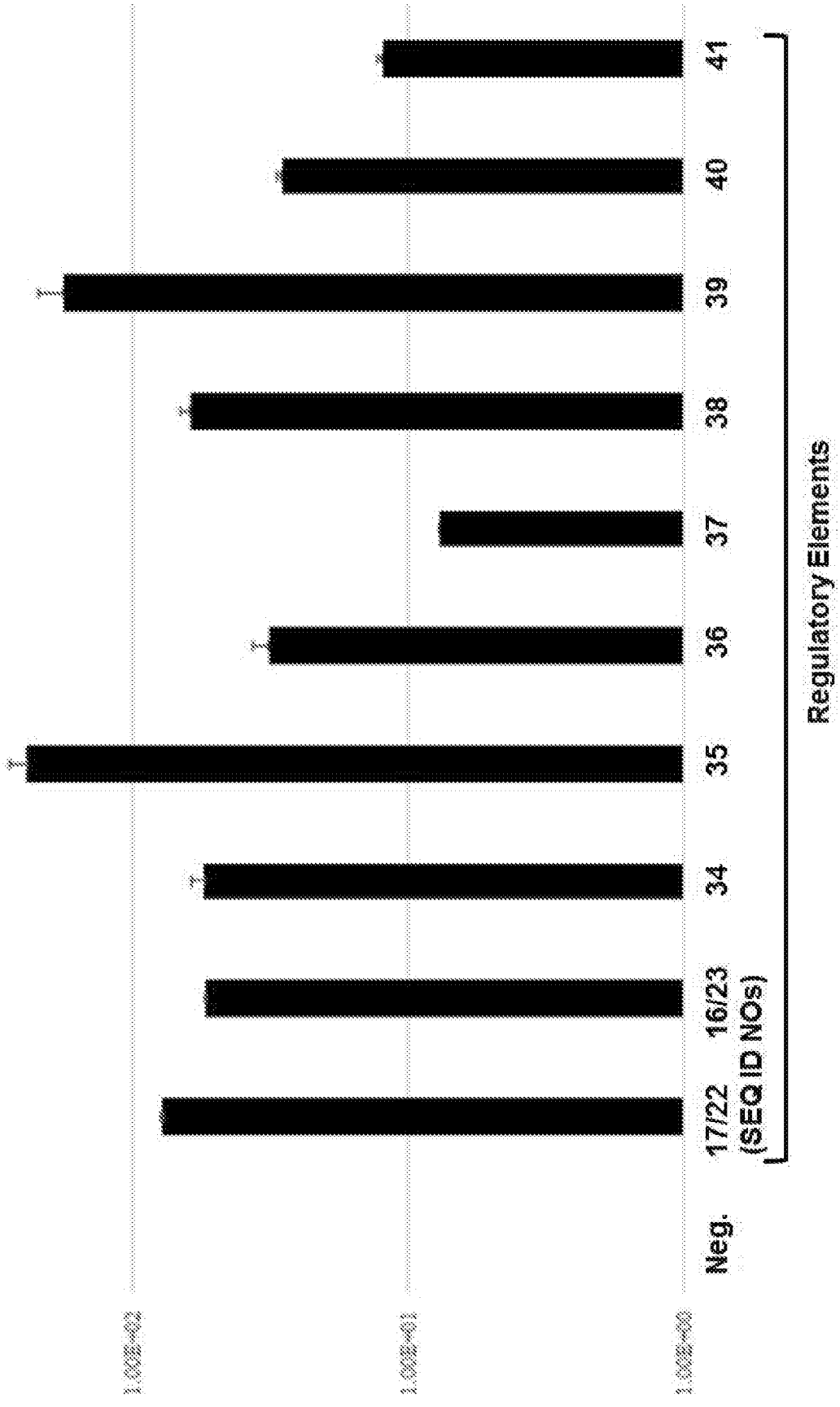

1.00E+02
1.00E+01
1.00E+00
Neg.
17/22
16/23
34
35
36
37
38
39
40
41
(SEQ ID NOs)
Regulatory Elements

FIG. 2A

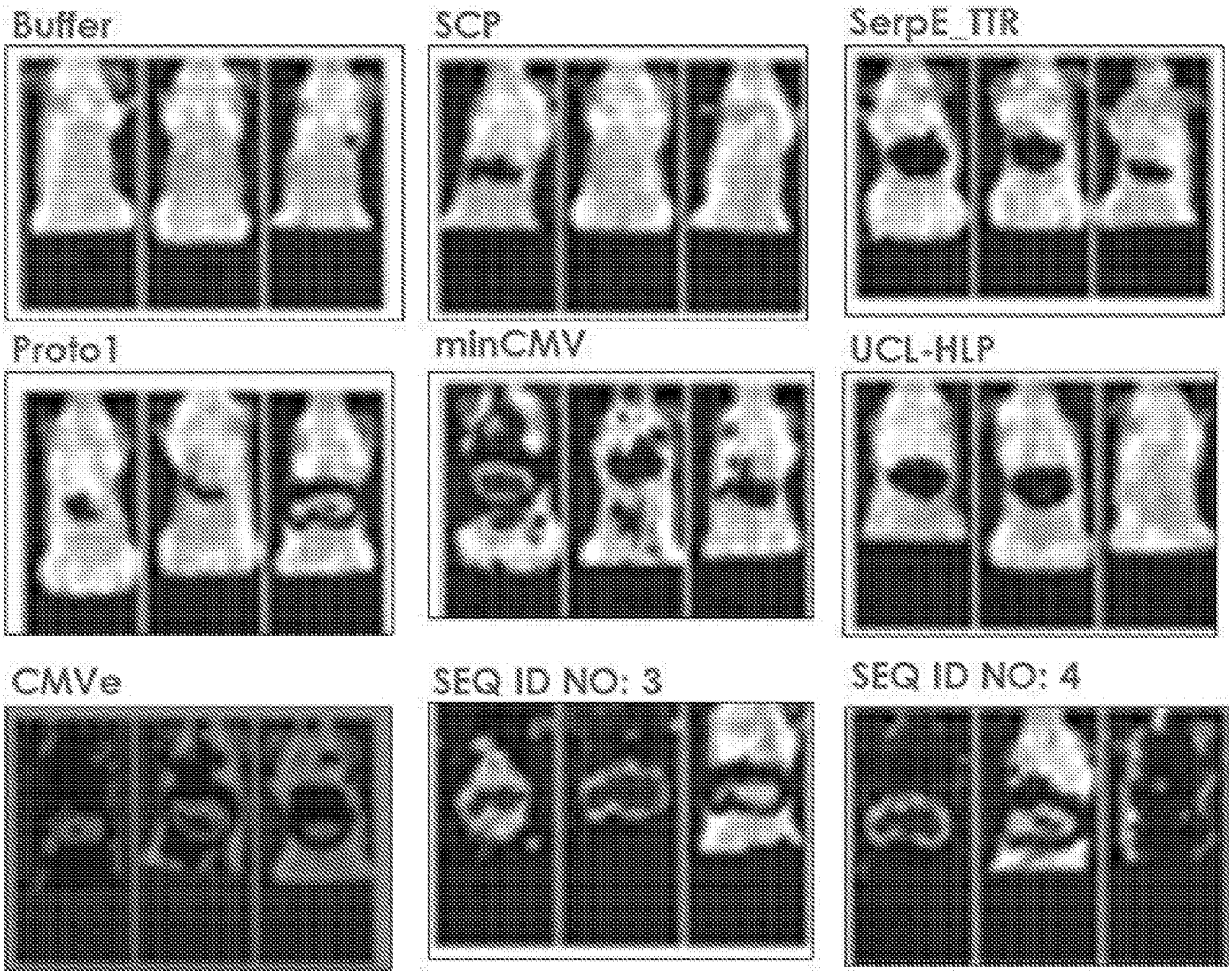

Buffer
SCP
SerpE_TTR
Proto1
minCMV
UCL-HLP
CMVe
SEQ ID NO: 3
SEQ ID NO: 4

FIG. 2B

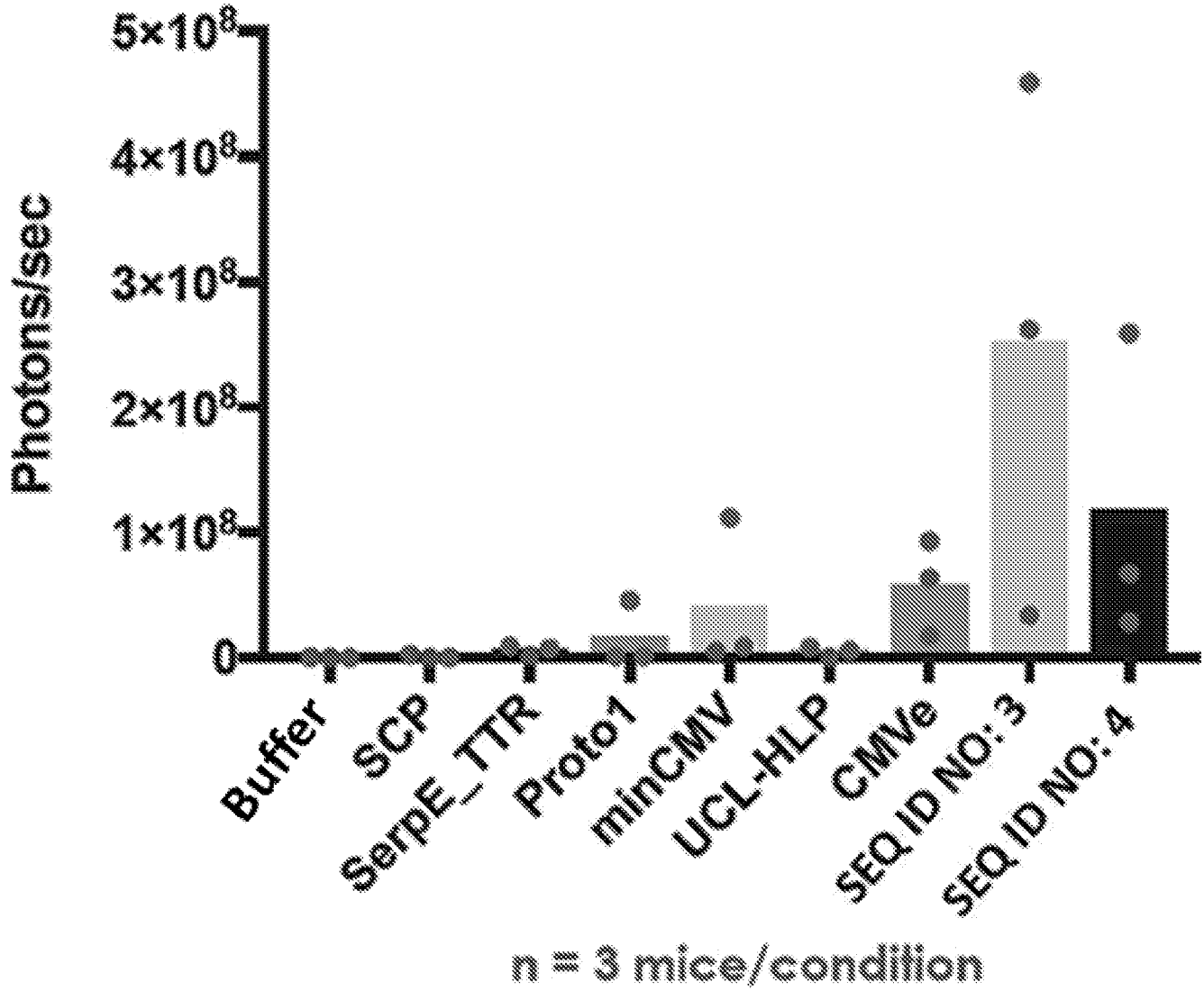

Photons/sec
5×10^8
4×10^8
3×10^8
2×10^8
1×10^8
0
Buffer
SCP
SerpE_TTR
Proto1
minCMV
UCL-HLP
CMVe
SEQ ID NO: 3
SEQ ID NO: 4
n = 3 mice/condition

FIG. 3A

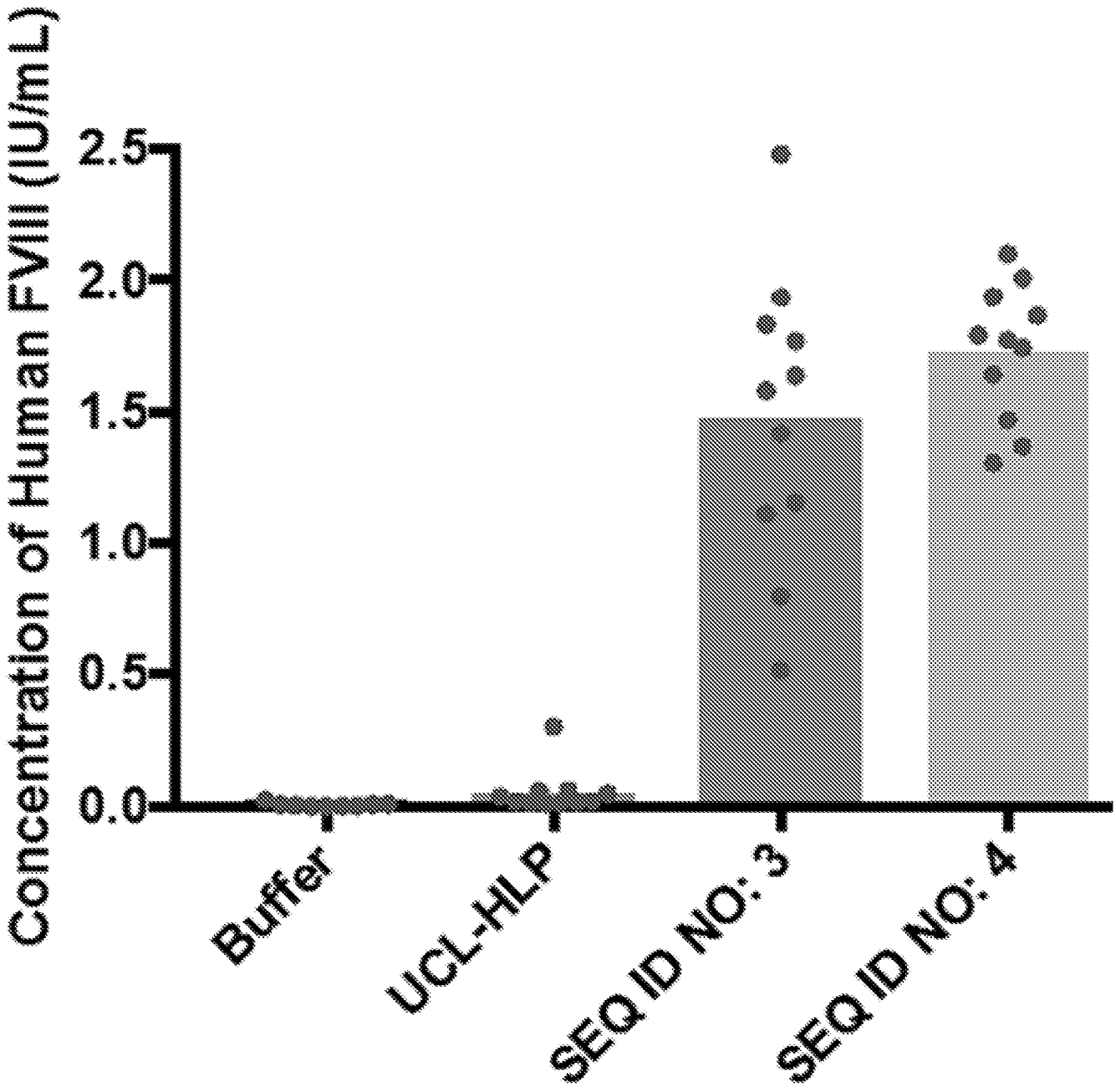

Concentration of Human FVIII (IU/mL)
2.5
2.0
1.5
1.0
0.5
0.0
Buffer
UCL-HLP
SEQ ID NO: 3
SEQ ID NO: 4

FIG. 3B

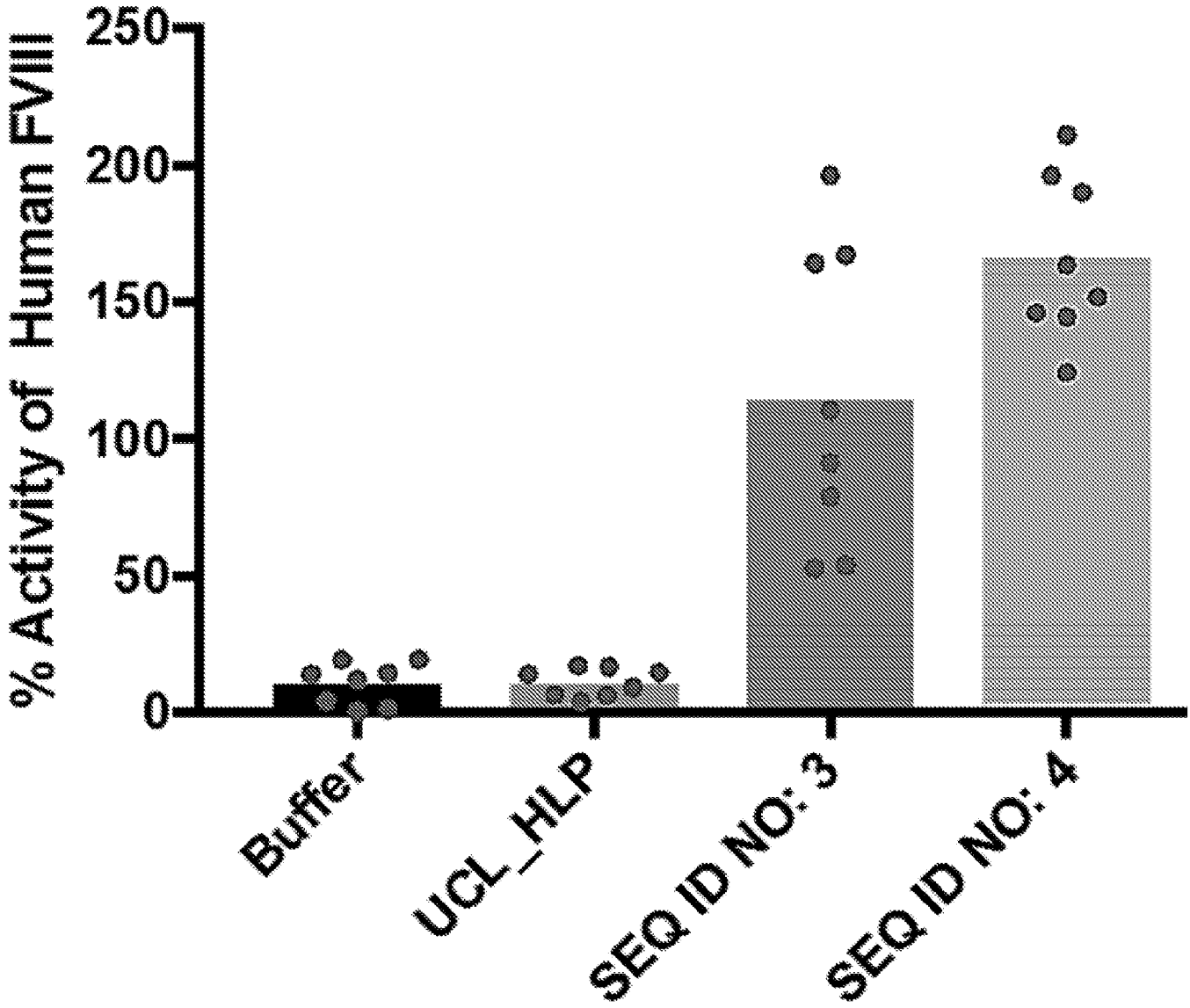

% Activity of Human FVIII
250
200
150
100
50
0
Buffer
UCL_HLP
SEQ ID NO: 3
SEQ ID NO: 4

FIG. 4A

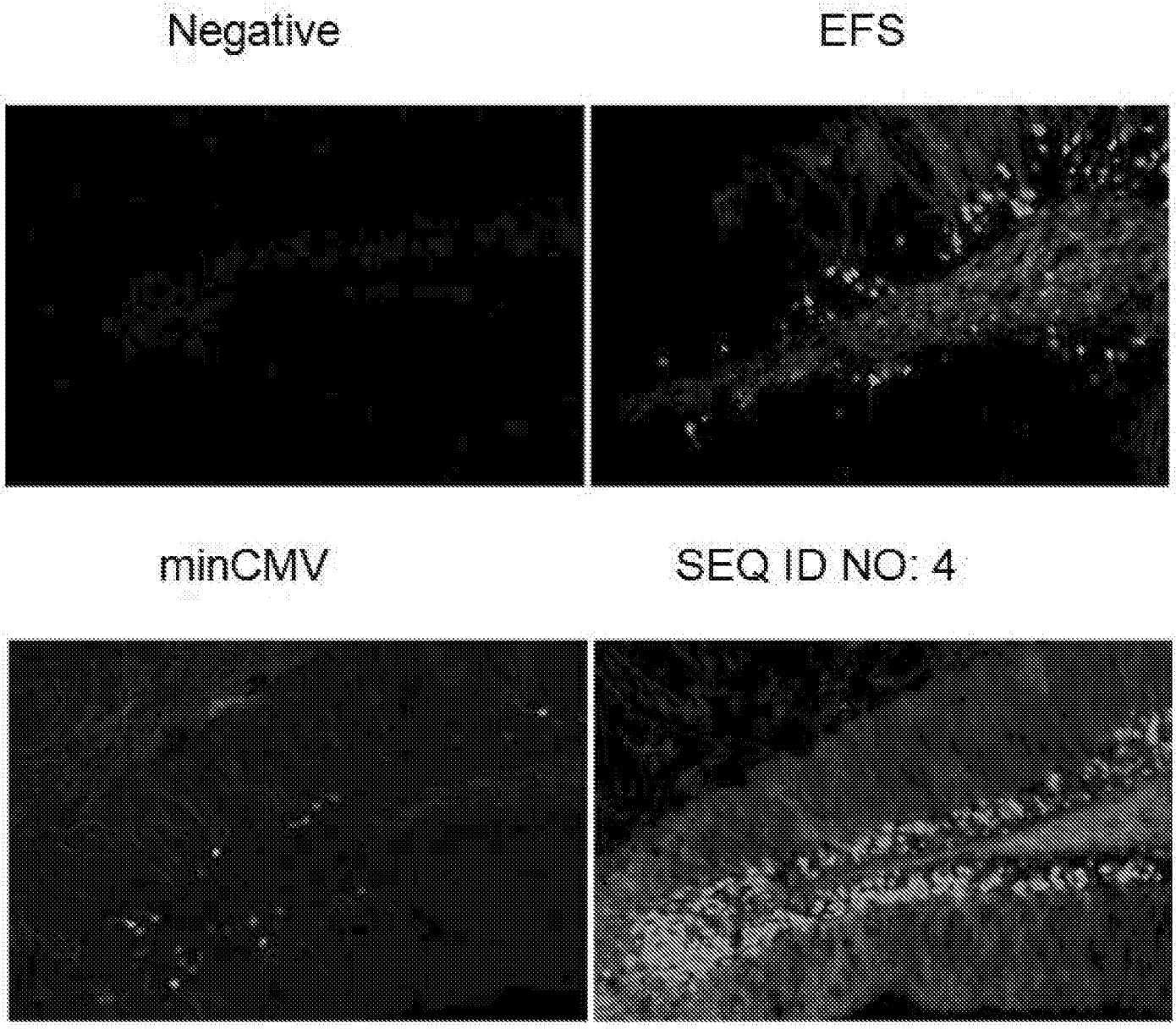


FIG. 4B

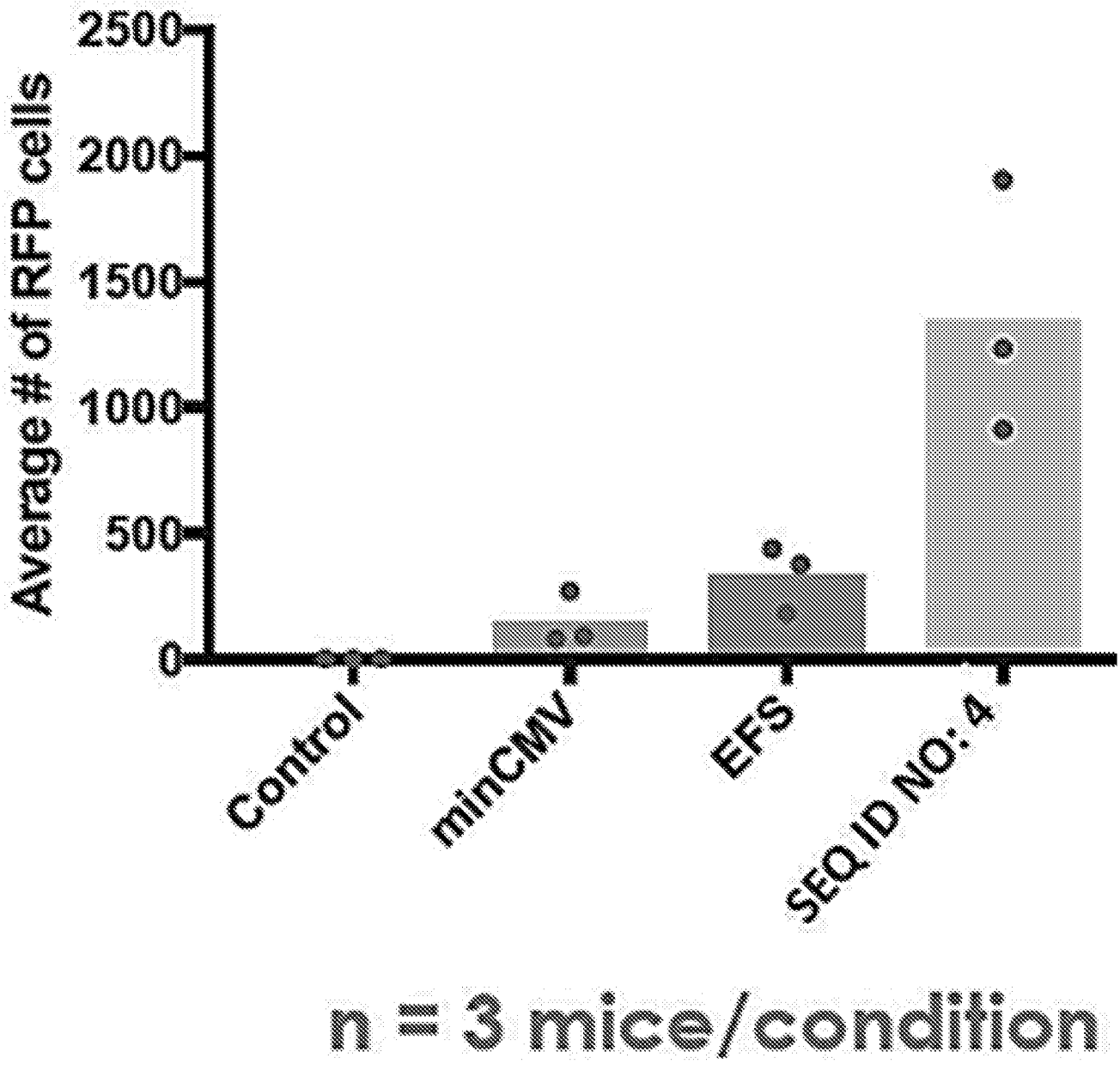

Average # of RFP cells
2500
2000
1500
1000
500
0
Control
minCMV
EFS
SEQ ID NO: 4
n = 3 mice/condition

FIG. 5A

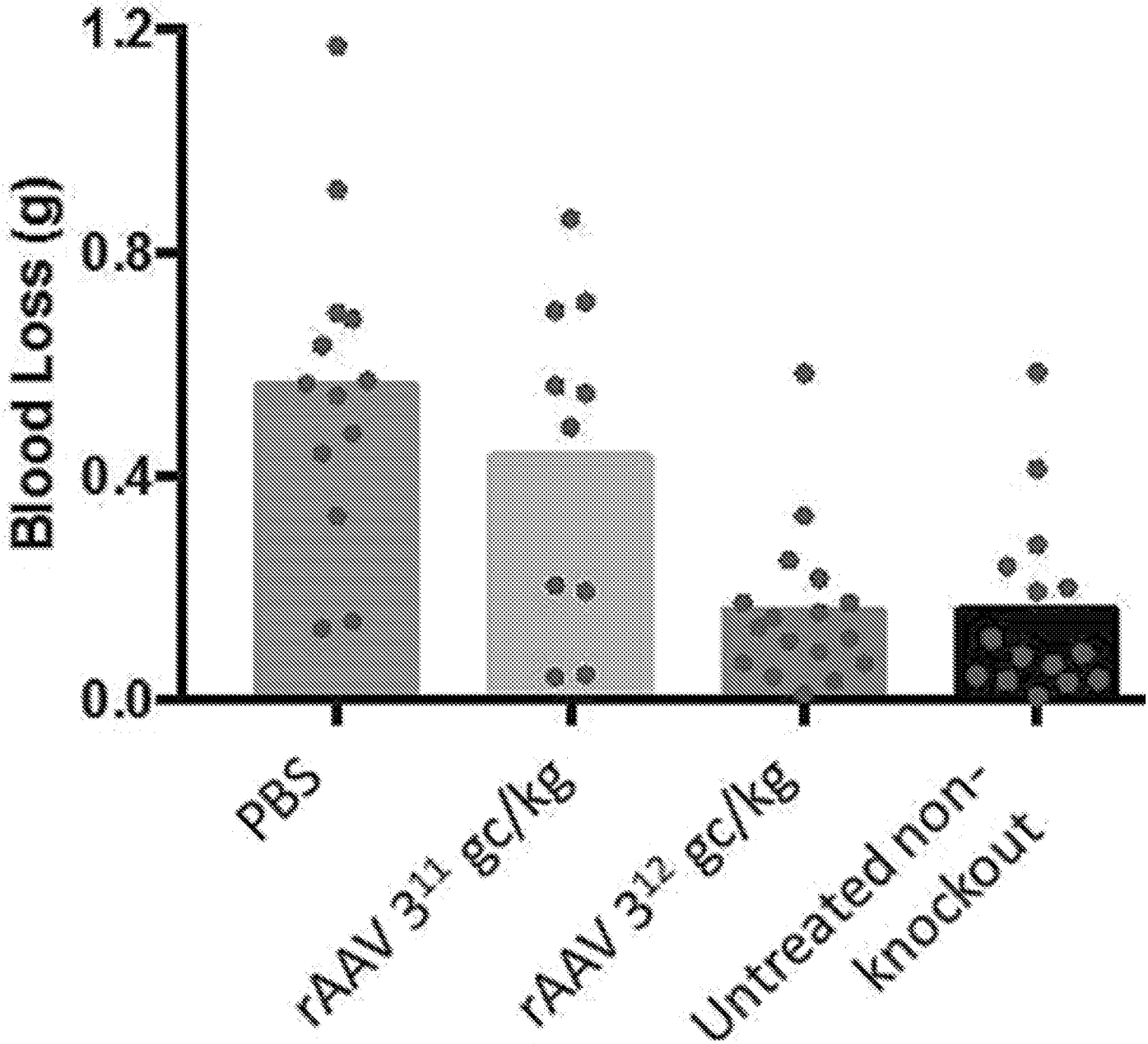

Blood Loss (g)
1.2
0.8
0.4
0.0
PBS
rAAV 3^11 gc/kg
rAAV 3^12 gc/kg
Untreated non-knockout

FIG. 5B

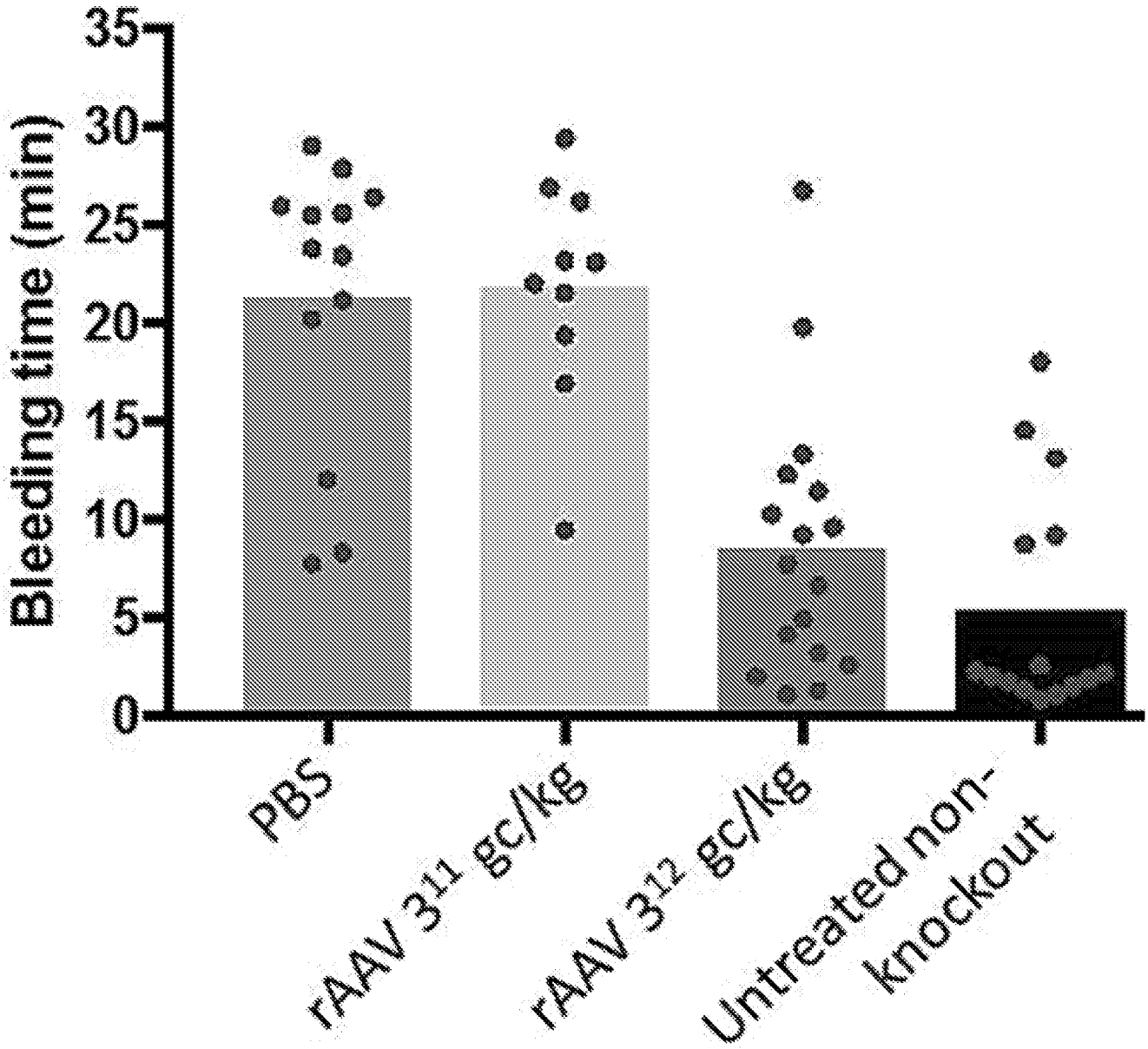

Bleeding time (min)
35
30
25
20
15
10
5
0
PBS
rAAV 3^11 gc/kg
rAAV 3^12 gc/kg
Untreated non-knockout

FIG. 6A

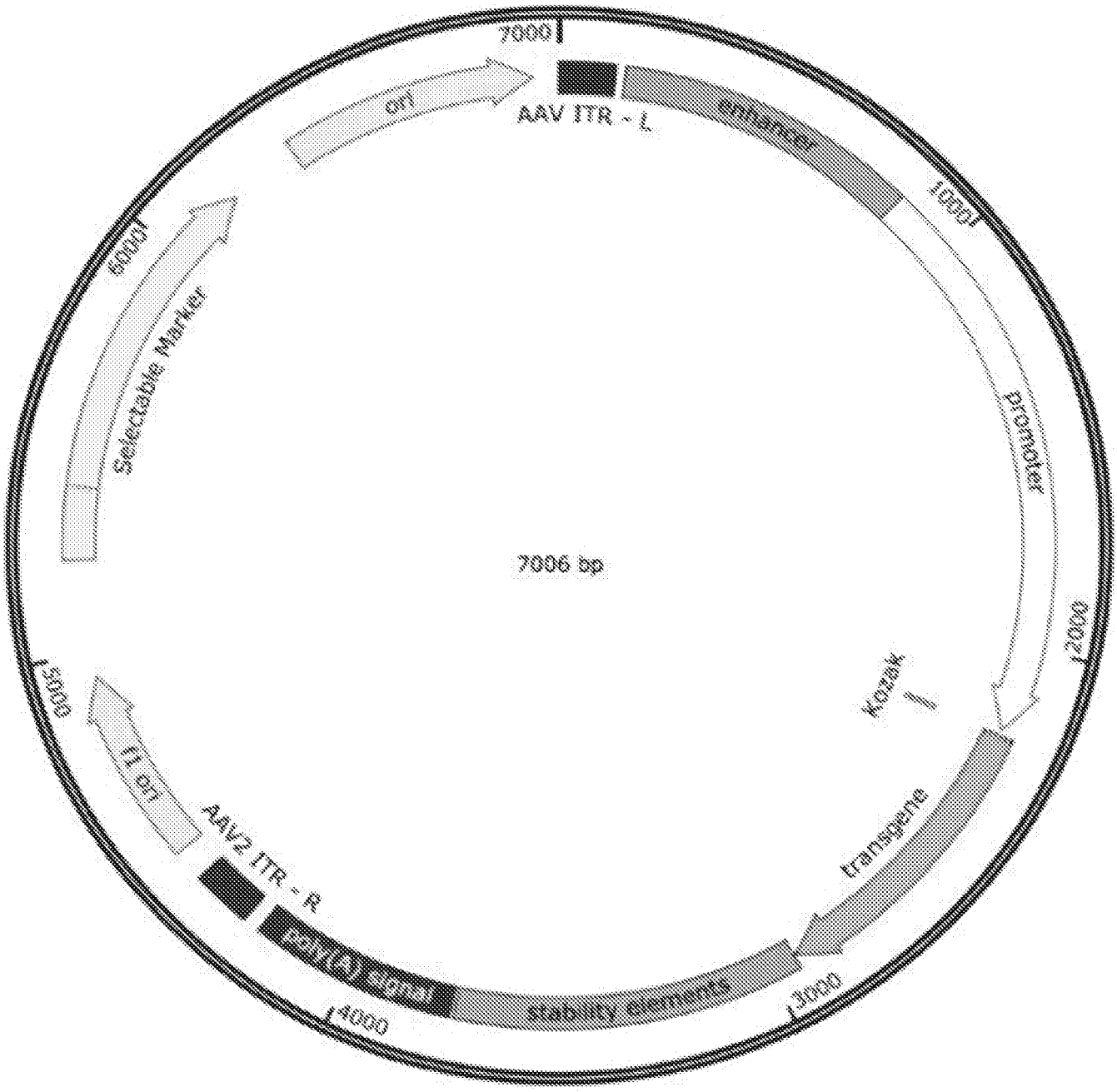
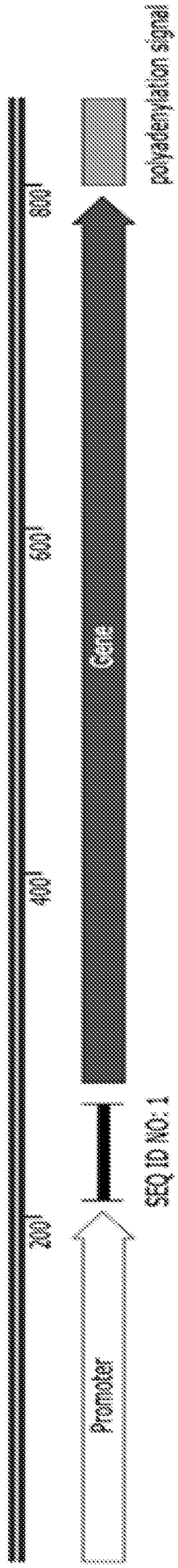

7000
ori
AAV ITR - L
Enhancer
1000
promoter
2000
Kozak
transgene
3000
stability elements
4000
EF1α
5000
f1 ori
AAV2 ITR - R
6000
Selectable Marker
7006 bp

FIG. 6B


polyadenylation signal
800
600
400
200
Gene
SEQ ID NO: 1
Promoter

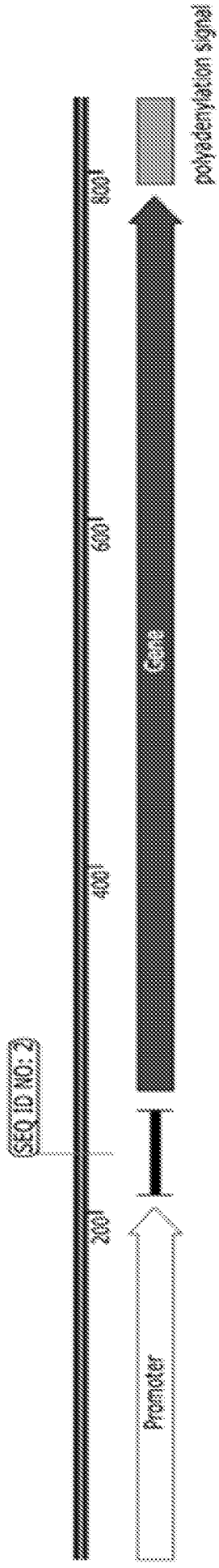

polyadenylation signal
800
600
400
SEQ ID NO: 2
200
Gene
Promoter

FIG. 6C

FIG. 7

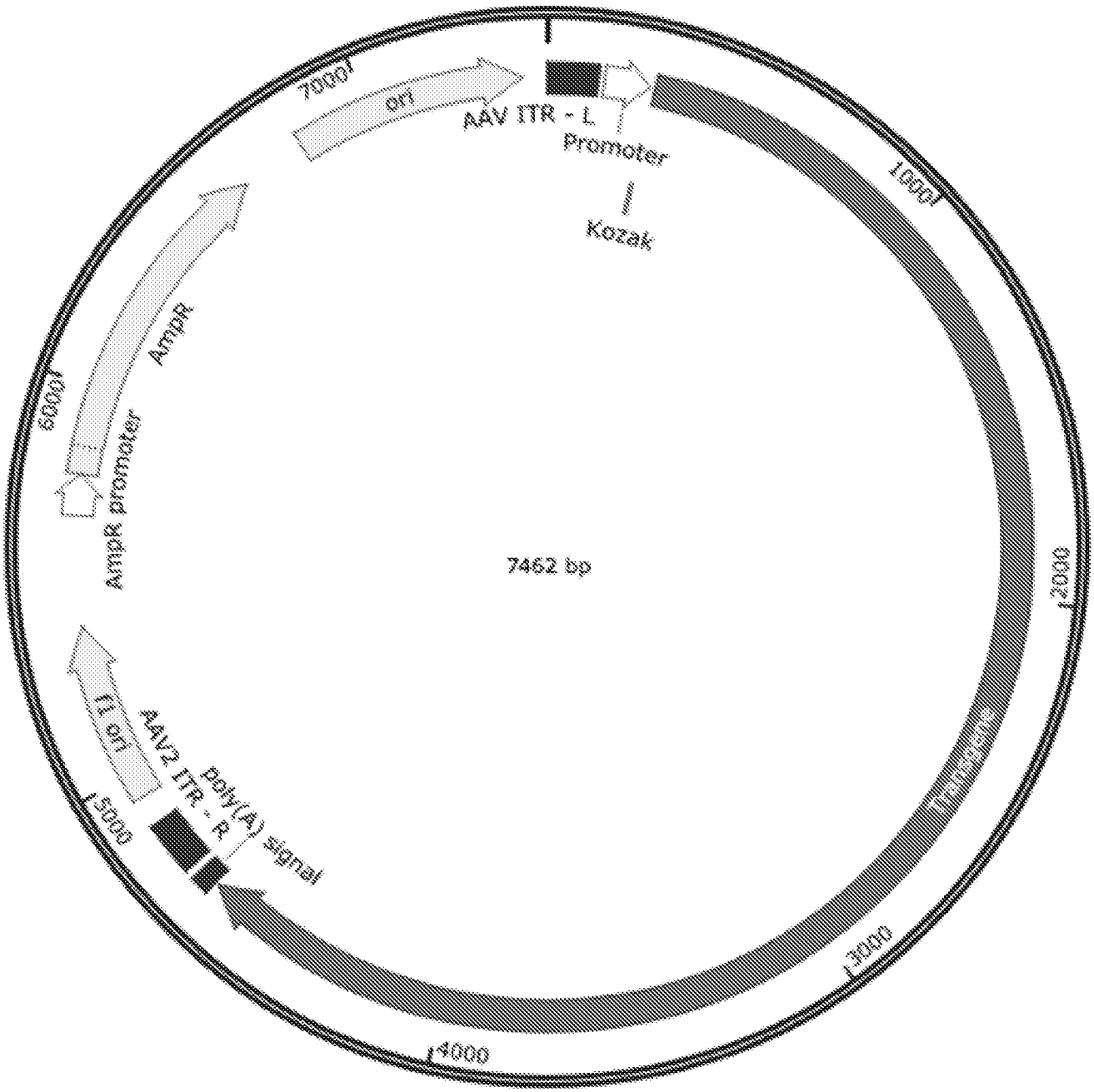

7000
ori
AAV ITR - L
Promoter
Kozak
1000
2000
3000
4000
5000
6000
AmpR
AmpR promoter
f1 ori
AAV2 ITR - R
poly(A) signal
7462 bp

FIG. 8

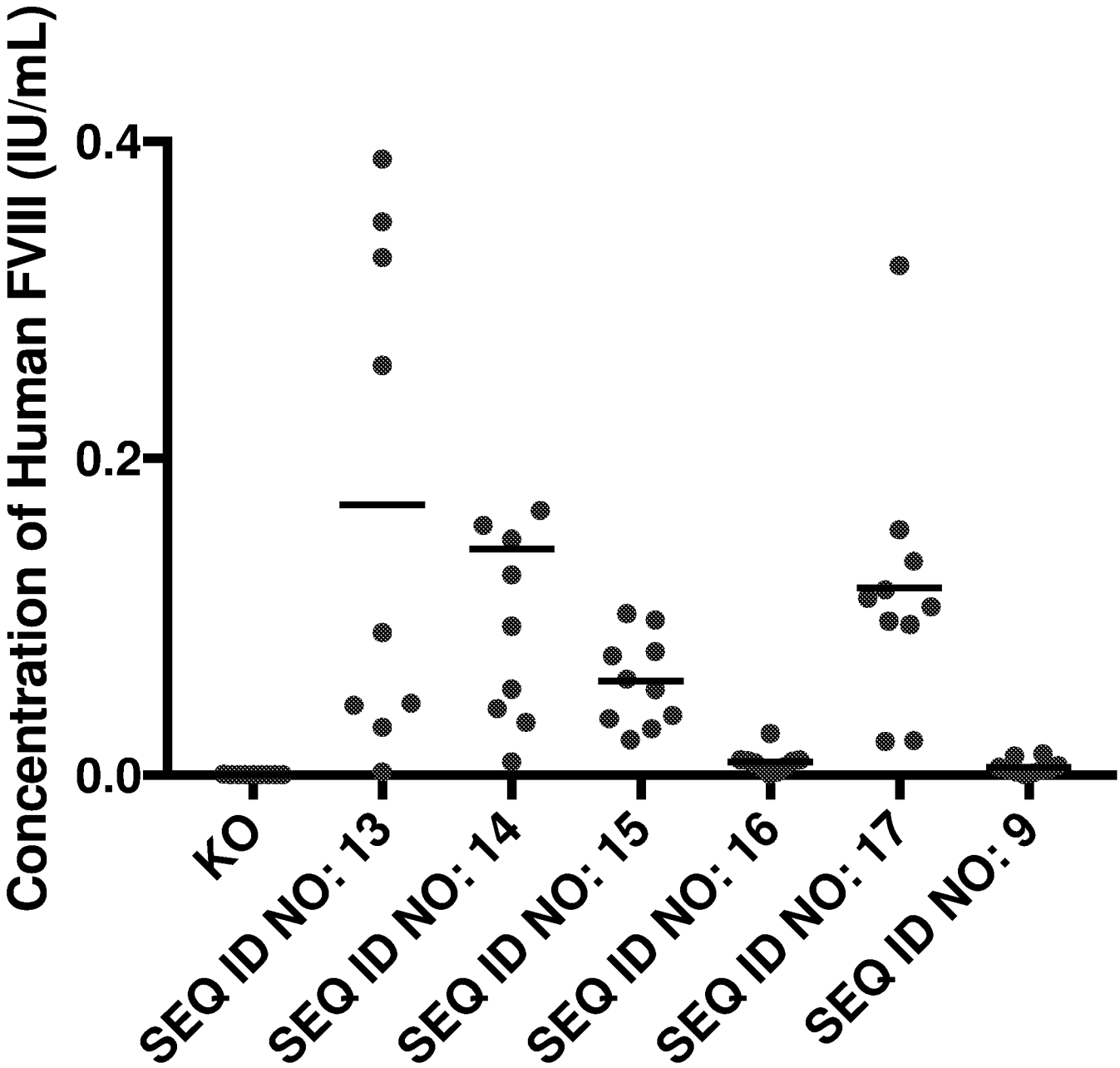

Concentration of Human FVIII (IU/mL)
0.4
0.2
0.0
KO
SEQ ID NO: 13
SEQ ID NO: 14
SEQ ID NO: 15
SEQ ID NO: 16
SEQ ID NO: 17
SEQ ID NO: 9

FIG. 9

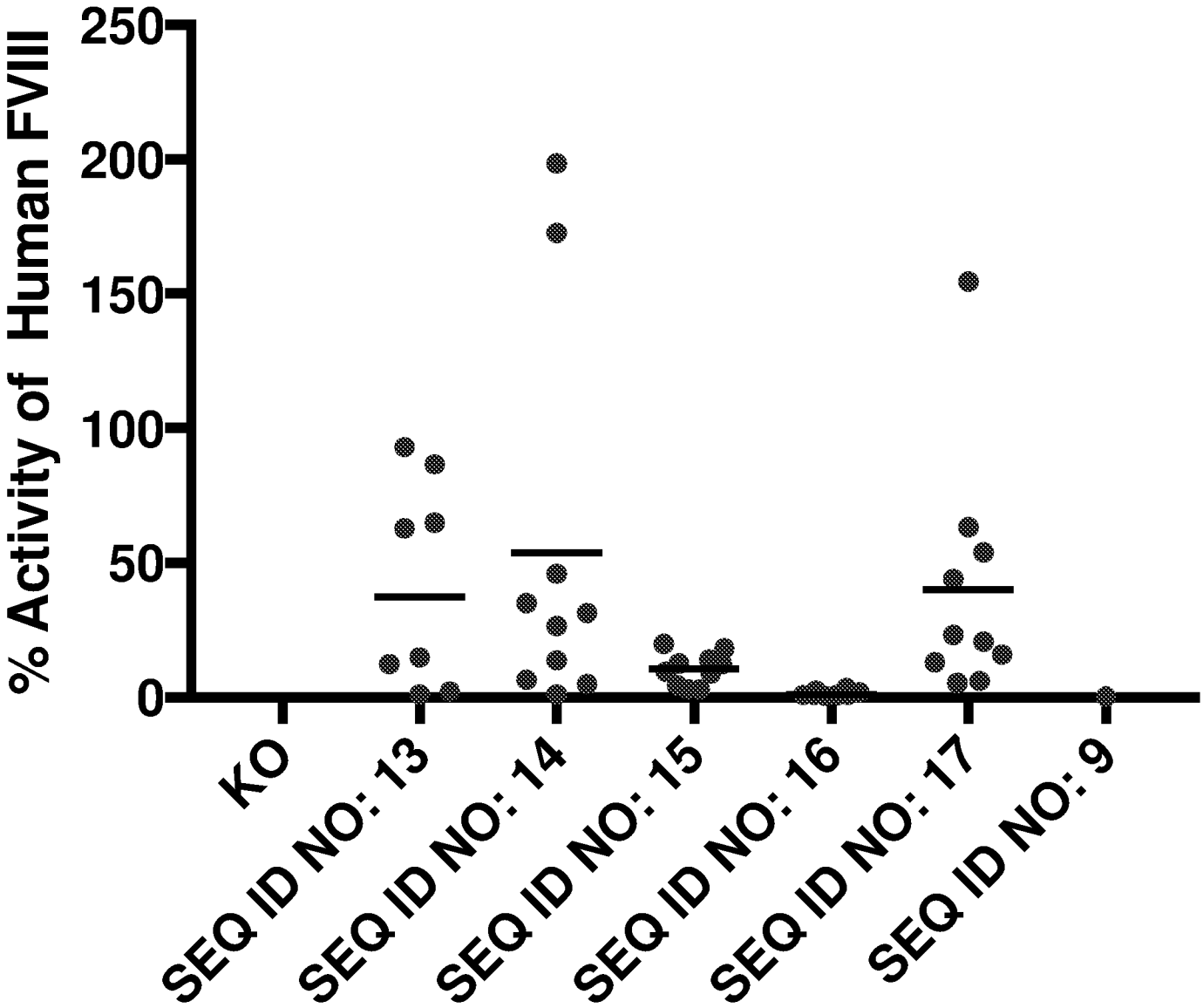

% Activity of Human FVIII
250
200
150
100
50
0
KO
SEQ ID NO: 13
SEQ ID NO: 14
SEQ ID NO: 15
SEQ ID NO: 16
SEQ ID NO: 17
SEQ ID NO: 9

FIG. 10

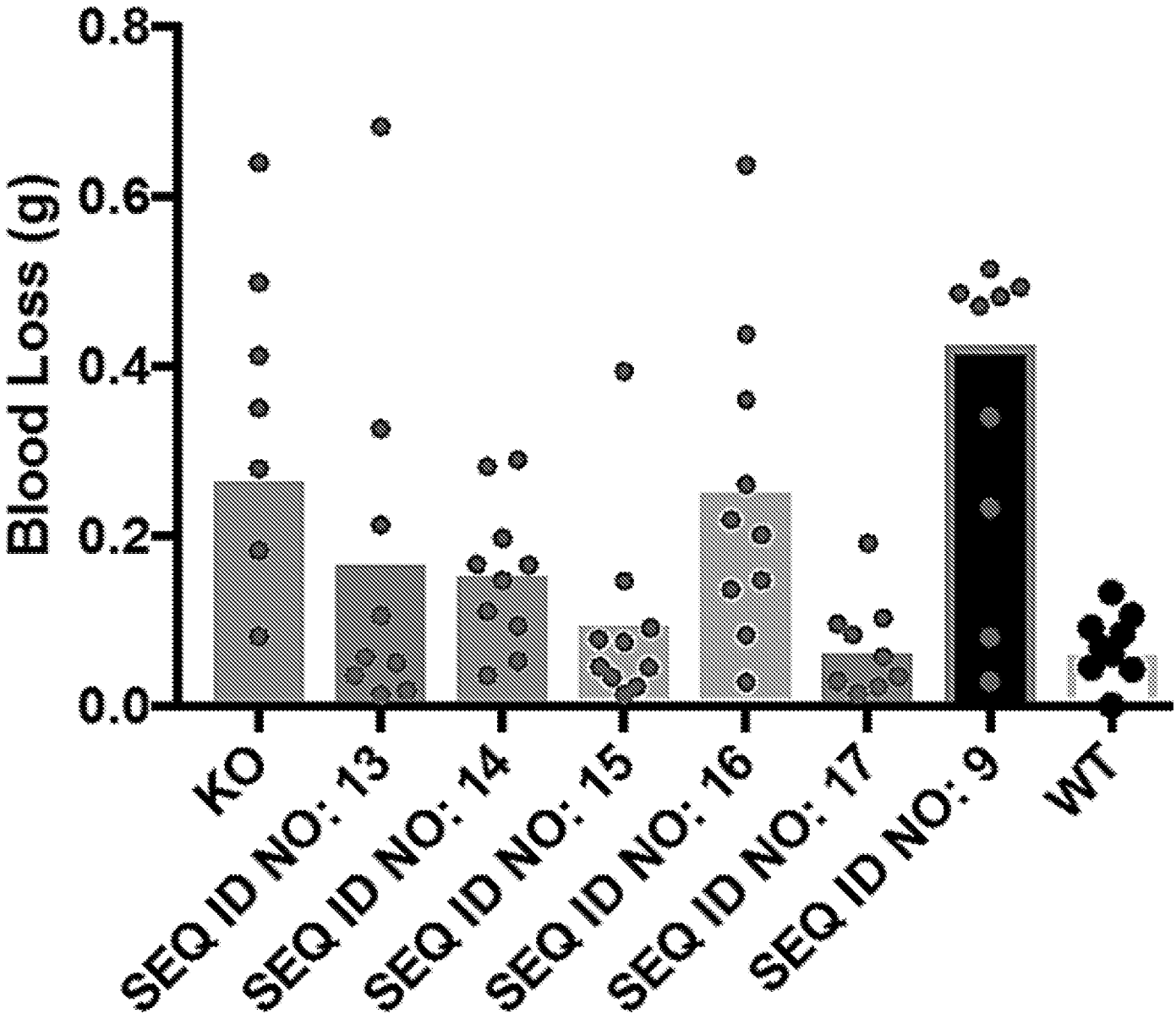

Blood Loss (g)
0.8
0.6
0.4
0.2
0.0
KO
SEQ ID NO: 13
SEQ ID NO: 14
SEQ ID NO: 15
SEQ ID NO: 16
SEQ ID NO: 17
SEQ ID NO: 9
WT

FIG. 11

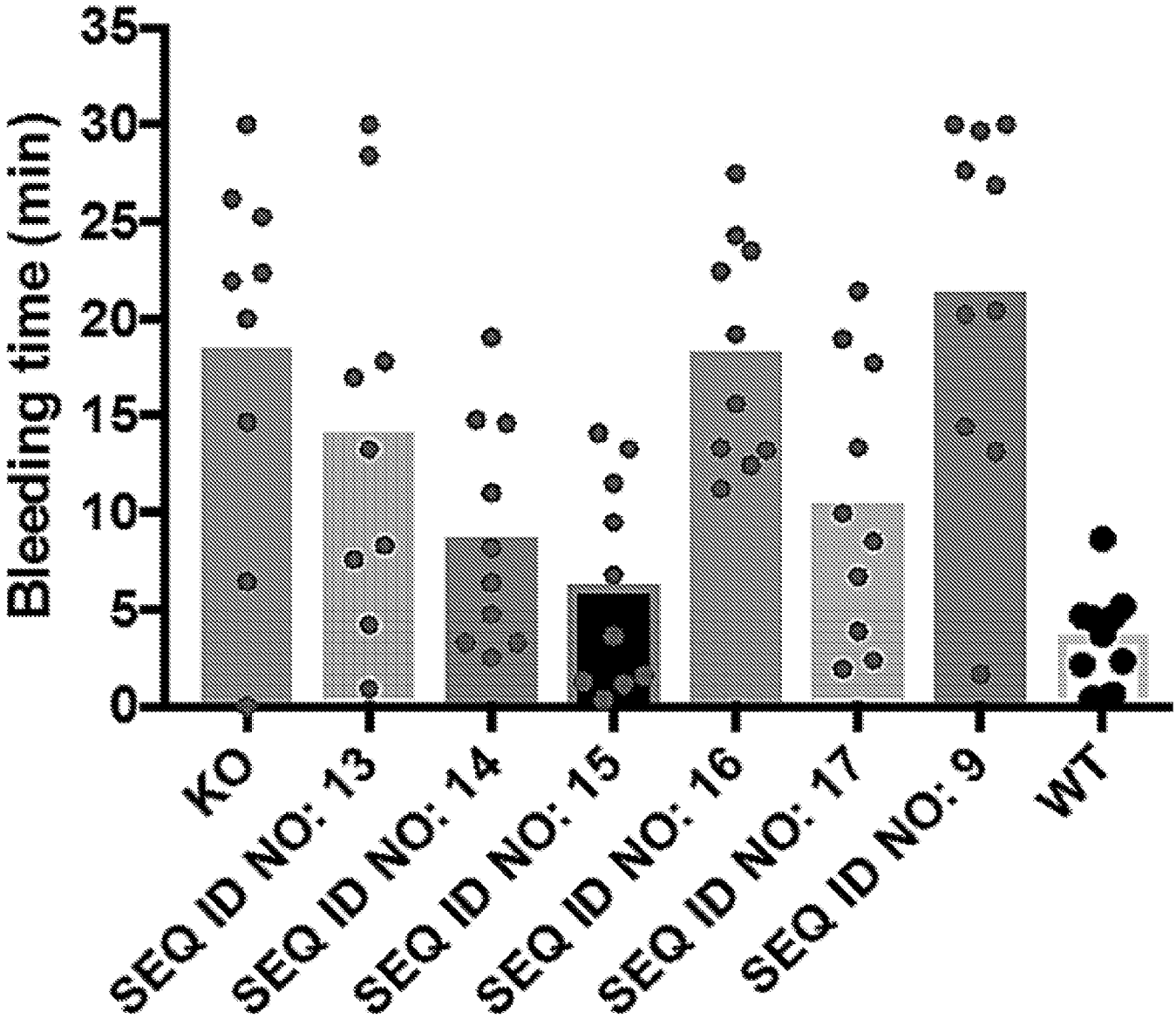

Bleeding time (min)
35
30
25
20
15
10
5
0
KO
SEQ ID NO: 13
SEQ ID NO: 14
SEQ ID NO: 15
SEQ ID NO: 16
SEQ ID NO: 17
SEQ ID NO: 9
WT

FIG. 12

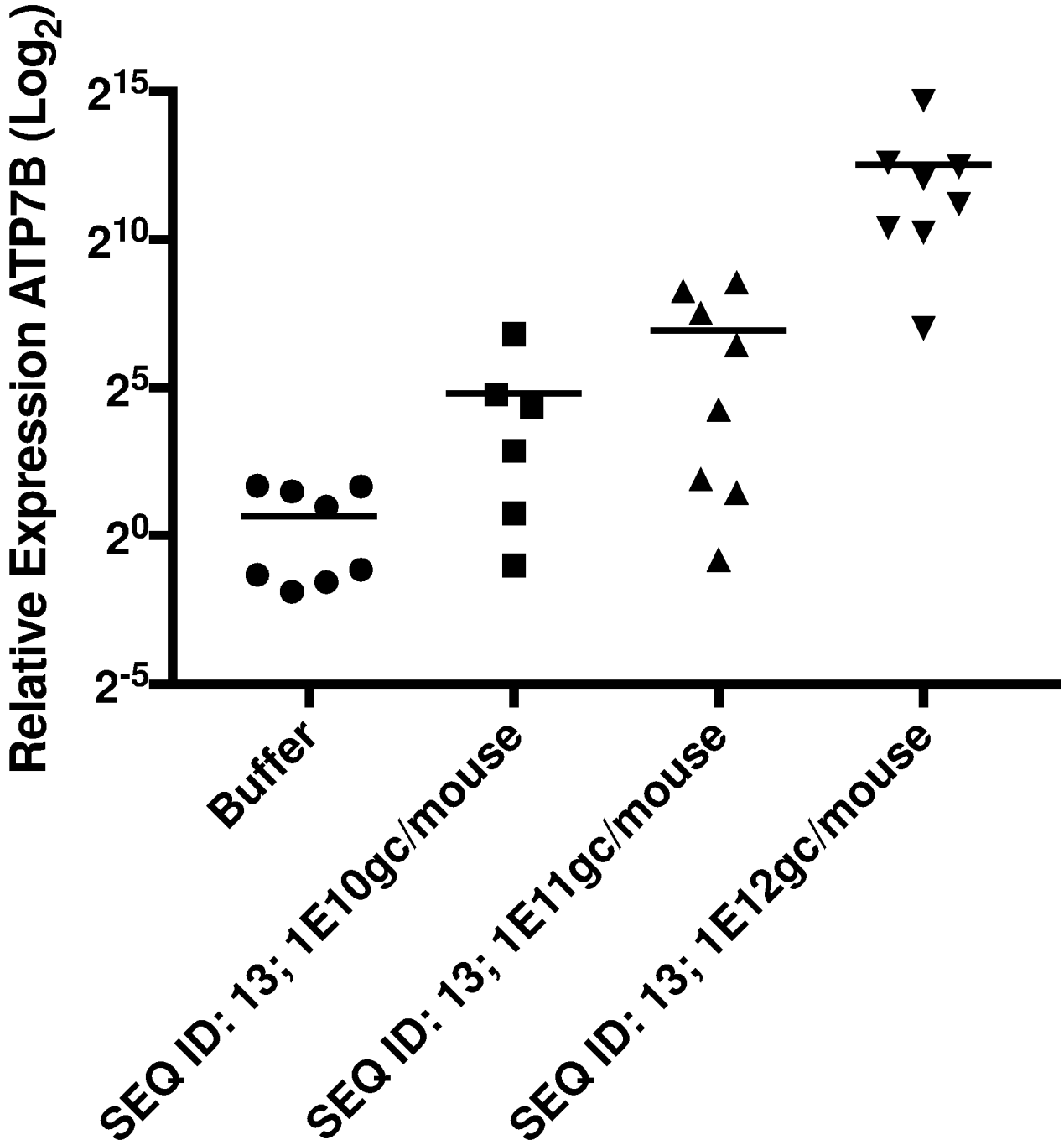
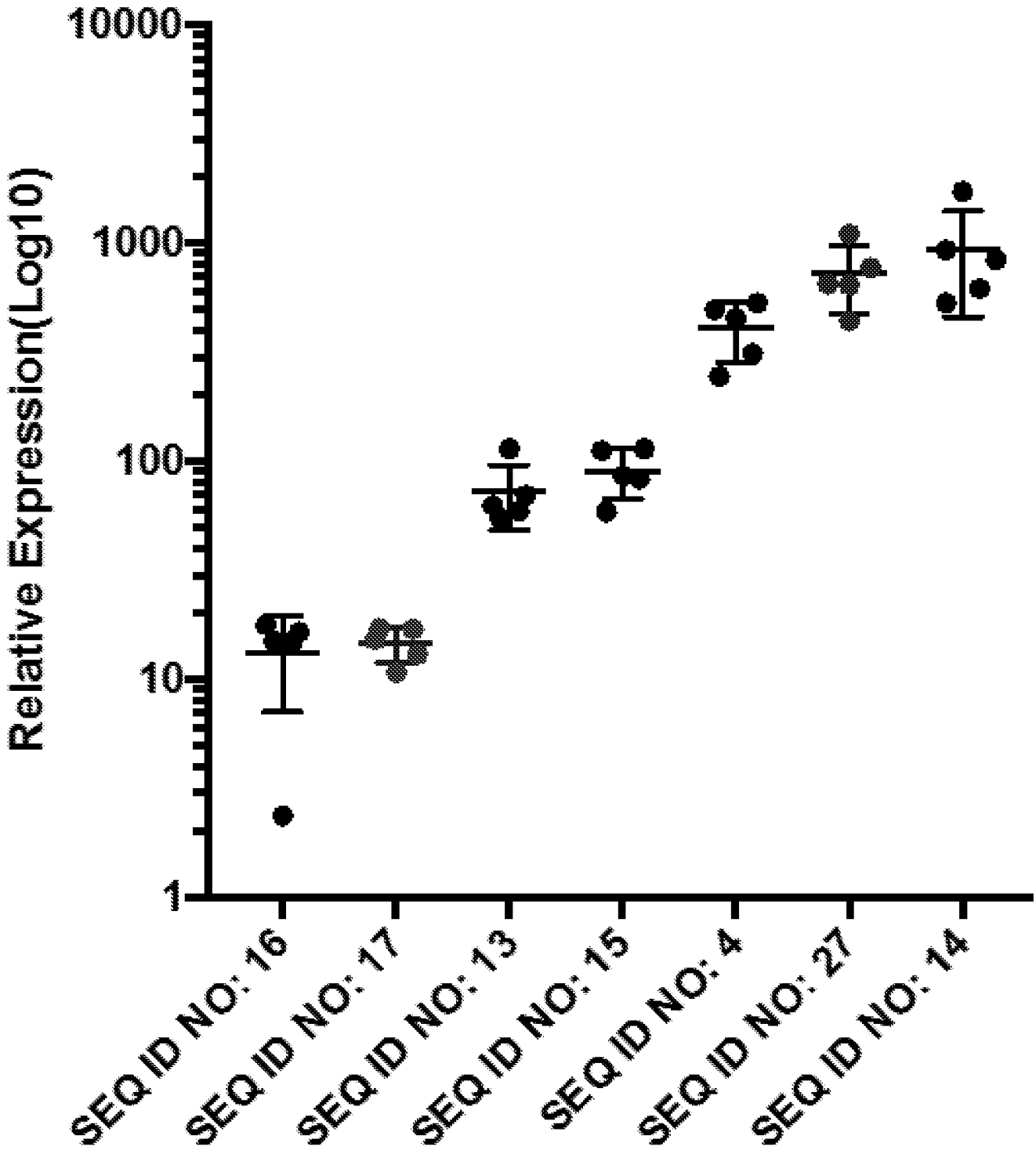

Relative Expression ATP7B (Log2)
2^15
2^10
2^5
2^0
2^-5
Buffer
SEQ ID: 13; 1E10gc/mouse
SEQ ID: 13; 1E11gc/mouse
SEQ ID: 13; 1E12gc/mouse

FIG. 13


10000
1000
100
10
1
Relative Expression(Log10)
SEQ ID NO: 16
SEQ ID NO: 17
SEQ ID NO: 13
SEQ ID NO: 15
SEQ ID NO: 4
SEQ ID NO: 27
SEQ ID NO: 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/33515

A. CLASSIFICATION OF SUBJECT MATTER
IPC - A61K 48/00; C12N 15/85, 15/861 (2018.01)
CPC - A61K 48/00, 48/0058; C12N 15/85, 15/86

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 2003/0017139 A1 (SOUZA et al.) January 23, 2003; abstract; figure 2; paragraphs [0001], [0014], [0016], [0017], [0019], [0024], [0025], [0027], [0028], [0033], [0036]	46, 48-52, 56, 88, 91, 92/88, 92/91, 97/88, 99/88, 99/91, 100, 113/88, 113/91, 121/88, 121/91, 122/88, 122/91 --- 57, 71/46, 71/51-52, 71/56, 72/71/46, 72/71/51-52, 72/71/56, 115/113/88, 115/113/91
X ----- Y	WO 2016/164609 A2 (GENZYME CORPORATION) October 13, 2016; figures 12A-B; paragraphs [0007], [0008], [0046], [0128], [0181], [0211]; claim 28	90, 95, 123 --- 71/46, 71/51-52, 71/56, 72/71/46, 72/71/51-52, 72/71/56
Y	CA 2985624 A1 (FUNDACION PARA LA INVESTIGACION MEDICA APLICADA) June 23, 2016; abstract; page 3, lines 18-19; claim 5	57, 115/113/88, 115/113/91

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
19 September 2018 (19.09.2018)

Date of mailing of the international search report
22 OCT 2018

Name and mailing address of the ISA/
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer
Shane Thomas
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/33515

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2009/0062135 A1 (DELFOUR et al.) March 5, 2009; paragraph [0095]	1-4, 7-12, 14/1-4, 14/7-12, 15/1-4, 15/7-12, 17/1-4, 17/7-12, 19/1-4, 19/7-12, 55, 71/54-55, 75, 77, 80-82, 83/74-75, 83/77, 83/80-82, 84-85
A	US 2005/0196754 A1 (DRMANAC et al.) September 8, 2005; paragraph [0006]	1-4, 7-12, 14/1-4, 14/7-12, 15/1-4, 15/7-12, 17/1-4, 17/7-12, 19/1-4, 19/7-12, 55, 71/54-55, 75, 77, 80-82, 83/74-75, 83/77, 83/80-82, 84-85

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/33515

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 108-112, 117-120
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-4, 7-12, 14/1-4, 14/7-12, 15/1-4, 15/7-12, 17/1-4, 17/7-12, 19/1-4, 19/7-12, 46-52, 54-57, 71/46-52, 71/54-57, 72/71/46-52, 72/71/54-57, 73-75, 77, 80-82, 83/74-75, 83/77, 83/80-82, 84-85, 88, 90-91, 92/88, 92/90-91, 93/88, 93/90-91, 95, 97/88, 97/90, 99/88, 99/90-91, 100, 113/88, 113/90-91, 115/113/88, 115/113/90-91, 121/88, 121/90-91, 122/88, 122/90-91, 123 ; SEQ ID NO: 1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US18/33515

-***-Continued from Box No. III Observations where unity of invention is lacking: -***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-107, 113-116, 121-126; SEQ ID NO: 1, an intronic sequence (regulatory element); ATP7B (transgene); Wilson's disease (disease or disorder) are directed toward an expression cassette and vector and methods associated therewith.

The expression cassettes, vectors and methods will be searched to the extent the encompass SEQ ID NO: 1 (first exemplary regulatory element sequence), an intronic sequence (first exemplary regulatory element type), ATP7B (first exemplary transgene); Wilson's disease (first exemplary disease or disorder) and a CMV promoter (first exemplary comparative control). Applicant is invited to elect additional regulatory element(s), with specified SEQ ID NO: for each, or with specified substitution(s) at specified site(s) of a SEQ ID NO:, such that the sequence of each elected species is fully specified (i.e. no optional or variable residues or substituents), and/or regulatory element type(s) and/or transgene(s) and/or specific disease(s) or disorder(s) and/or comparative control(s) to be searched. Additional regulatory element sequence(s), and/or regulatory element type(s) and/or transgene(s) and/or specific disease(s) or disorder(s) and/or comparative control(s) will be searched upon the payment of additional fees. It is believed that claims 1-4 (each in-part), 7-12 (each in-part), 14 (in-part), 15 (in-part), 17 (in-part), 19 (in-part), 46, 47 (in-part), 48-52, 54-57 (each in-part), 71-75 (each in-part), 77 (in-part), 80-85 (each in-part), 88 (in-part), 90-92, 93 (in-part), 95-97, 98 (in-part), 99 (in-part), 100 (in-part), 113 (in-part), 115 (in-part), 121 (in-part), 122 (in-part), and 123 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1 (regulatory element sequence), an intronic sequence (regulatory element type), ATP7B (transgene); Wilson's disease (disease or disorder) and a CMV promoter (comparative control). Applicants must specify the claims that encompass any additionally elected regulatory element sequence(s), and/or regulatory element type(s) and/or transgene(s) and/or specific disease (s) or disorder(s) and/or comparative control(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be SEQ ID NO: 2 (regulatory element sequence).

No technical features are shared between the regulatory element sequences and/or types and/or transgenes and/or diseases or disorders and/or comparative controls of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features including: an expression cassette comprising a regulatory element operably linked to a therapeutic transgene not found in context with the regulatory element in a cell or in vivo; a method of treating a liver disease or condition, comprising administering a gene therapy comprising the expression cassette; a method of treating a haploinsufficiency or a genetic defect, comprising administering the expression cassette; an AAV expression cassette or vector comprising a regulatory element of no more than 120 bp operably linked to a transgene of at least 3kb, wherein the regulatory element results in increased transgene expression as compared to a control; a method of producing a recombinant protein, the method comprising operably linking a sequence encoding the protein with a sequence; an expression vector comprising a human-derived regulatory element operably linked to a transgene, wherein a protein encoded by the transgene has (i) a concentration 0.1 IU/mL as measured by an ELISA assay configured to detect the transgene; or (ii) .g.t. 10% activity as measured by a Coatest assay; these shared technical features are previously disclosed by WO 2016/064609 A2 to Genzyme Corporation (hereinafter 'Genzyme') in light of evidence provided by the article 'Multiyear therapeutic benefit of AAV serotypes 2, 6, and 8 delivering factor VIII to hemophilia A mice and dogs' by Jiang et al. (hereinafter 'Jiang') and in light of evidence provided by GenBank Accession K01740 (hereinafter 'K01740').

Genzyme discloses an expression cassette (an AAV particle comprising a heterologous transgene operably linked to a promoter and an intron (an expression cassette); paragraphs [0007], [0008]) comprising a regulatory element (comprising a promoter and an intron (a regulatory element); paragraph [0008]) operably linked to a therapeutic transgene (operably linked to a therapeutic transgene; paragraph [0008]) not found in context with the regulatory element in a cell or in vivo (wherein the transgene in heterologous (not found in context with the regulatory element in a cell or in vivo); paragraphs [0007], [0008]); a method of treating a liver disease or condition (a method of treating hemophilia A (a liver disease or condition); paragraph [0030]), comprising administering a gene therapy comprising the expression cassette (comprising administering a gene therapy comprising the expression cassette; paragraphs [0007], [0008], [0030]); a method of treating a haploinsufficiency or a genetic defect (a method of treating hemophilia A (a haploinsufficiency or a genetic defect); paragraph [0030]), comprising administering the expression cassette (comprising administering the expression cassette; paragraphs [0007], [0008], [0030]); an AAV expression cassette or vector (an AAV expression cassette or vector; paragraphs [0007], [0008]) comprising a regulatory element of no more than 120 bp (comprising an intron (comprising a regulatory element of no more than 120 bp); paragraphs [0008], [0170], wherein the hybrid intron comprises 106 base pairs [see Jiang, page 108, first column, second paragraph]) operably linked to a transgene of at least 3 kb (operably linked to a transgene encoding human Factor VIII (operably linked to a transgene of at least 3 kb); paragraph [0124]; wherein GenBank Accession No. AAA52484 is encoded by Genbank Accession K01740, which discloses a sequence of 9 kb), wherein the regulatory element results in increased transgene expression as compared to a control (wherein the regulatory element results in increased transgene expression as compared to a control; paragraphs [0181], [0213]; Figures 10A, 12A); a method of producing a recombinant protein (a method of producing a recombinant protein; paragraphs [0006], [0007], [0123]), the method comprising operably linking a sequence encoding the protein with a sequence (the method comprising operably linking a sequence encoding the protein with a sequence; paragraphs [0007], [0008], [0066]); an expression vector comprising a human-derived regulatory element (an expression vector comprising a GUSB or PGK-1 promoter (a human-derived regulatory element); paragraph [0128]) operably linked to a transgene (operably linked to a transgene; paragraphs [0007], [0008]), wherein a protein encoded by the transgene has .g.t. 10% activity as measured by a Coatest assay (wherein a protein encoded by the transgene has .g.t. 10% activity as measured by a Coatest assay; paragraph [0046]; Fig 10B).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Genzyme reference, unity of invention is lacking.