CLAIMS

WHAT IS CLAIMED IS:

1. A system comprising:

a deoxyribonucleic acid (DNA) endonuclease or nucleic acid encoding said DNA endonuclease;

a guide RNA (gRNA) comprising a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, 30, 18-20, 23-27, 29, 31-44, and 104, or nucleic acid encoding the gRNA; and

a donor template comprising a nucleic acid sequence encoding a gene-of-interest (GOI) or functional derivative thereof.

2. The system of claim 1, wherein the gRNA comprises a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, and 30.

3. The system of claim 2, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 22.

4. The system of claim 2, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 21.

5. The system of claim 2, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 28.

6. The system of claim 2, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 30.

7. The system of any one of claims 1-6, wherein said DNA endonuclease recognizes a protospacer adjacent motif (PAM) having the sequence NGG or NNGG, wherein N is any nucleotide, or a functional derivative thereof.

8. The system of any one of claims 1-7, wherein said DNA endonuclease is a type II Cas endonuclease or a functional derivative thereof.

9. The system of any one of claims 1-8, wherein said DNA endonuclease is Cas9.

10. The system of any one of claims 1-9, wherein the nucleic acid encoding said DNA endonuclease is codon optimized for expression in a host cell.

11. The system of any one of claims 1-10, wherein the nucleic acid sequence encoding the GOI or functional derivative thereof is codon optimized for expression in a host cell.

12. The system of any one of claims 1-11, wherein the GOI encodes a polypeptide selected from the group consisting of a therapeutic polypeptide and a prophylactic polypeptide.

13. The system of any one of claims 1-11, wherein the GOI encodes a protein selected from the group consisting of Factor VIII (FVIII) protein, Factor IX (FIX) protein, alpha-1-antitrypsin, Factor XIII (FXIII) protein, Factor VII (FVII) protein, Factor X (FX) protein, Protein C, serine protease inhibitor G1 (serpin G1), or a functional derivative of any thereof.

14. The system of claim 13, wherein the GOI encodes a FVIII protein or a functional derivative of any thereof.

15. The system of claim 13, wherein the GOI encodes a FIX protein or a functional derivative of any thereof.

16. The system of claim 13, wherein the GOI encodes serpin G1 protein or a functional derivative of any thereof.

17. The system of any one of claims 1-16, wherein the nucleic acid encoding said DNA endonuclease is a deoxyribonucleic acid (DNA).

18. The system of any one of claims 1-16, wherein the nucleic acid encoding said DNA endonuclease is a ribonucleic acid (RNA).

19. The system of claim 18, wherein the RNA encoding said DNA endonuclease is an mRNA.

20. The system of any one of claims 1-19, wherein the donor template is encoded in an AAV vector.

21. The system of claim 20, wherein the donor template comprises a donor cassette comprising the nucleic acid sequence encoding the GOI or functional derivative, and wherein the donor cassette is flanked on one or both sides by a gRNA target site.

22. The system of claim 21, wherein the donor cassette is flanked on both sides by a gRNA target site.

23. The system of claim 21 or 22, wherein the gRNA target site is a target site for a gRNA in the system.

24. The system of claim 23, wherein the gRNA target site of the donor template is the reverse complement of a genomic gRNA target site for a gRNA in the system.

25. The system of any one of claims 1-24, wherein said DNA endonuclease or nucleic acid encoding the DNA endonuclease is formulated in a liposome or lipid nanoparticle.

26. The system of claim 25, wherein said liposome or lipid nanoparticle also comprises the gRNA.

27. The system of any one of claims 1-26, comprising the DNA endonuclease precomplexed with the gRNA, forming a ribonucleoprotein (RNP) complex.

28. A method of editing a genome in a cell, the method comprising:

providing the following to the cell:

(a) a gRNA comprising a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, 30, 18-20, 23-27, 29, 31-44, and 104, or nucleic acid encoding the gRNA;

(b) a DNA endonuclease or nucleic acid encoding the DNA endonuclease; and

(c) a donor template comprising a nucleic acid sequence encoding a gene-of-interest (GOI) or functional derivative.

29. The method of claim 28, wherein the gRNA comprises a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, and 30.

30. The method of claim 29, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 21.

31. The method of claim 30, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 22.

32. The method of claim 29, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 28.

33. The method of claim 29, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 30.

34. The method of any one of claims 28-33, wherein said DNA endonuclease recognizes a protospacer adjacent motif (PAM) having the sequence NGG or NNGG, wherein N is any nucleotide, or a functional derivative thereof.

35. The method of any one of claims 28-34, wherein said DNA endonuclease is a type II Cas endonuclease or a functional derivative thereof.

36. The method of any one of claims 28-35, wherein said DNA endonuclease is Cas9.

37. The method of any one of claims 28-36, wherein the nucleic acid encoding said DNA endonuclease is codon optimized for expression in the cell.

38. The method of any one of claims 28-37, wherein the nucleic acid sequence encoding the GOI or functional derivative thereof is codon optimized for expression in the cell.

39. The method of any one of claims 28-38, wherein the GOI encodes a polypeptide selected from the group consisting of a therapeutic polypeptide and a prophylactic polypeptide.

40. The method of any one of claims 28-38, wherein the GOI encodes a protein selected from the group consisting of FVIII protein, FIX protein, alpha-1-antitrypsin, FXIII protein, FVII protein, FX protein, Protein C, serpin G1, or a functional derivative of any thereof.

41. The method of any one of claims 28-40, wherein the nucleic acid encoding said DNA endonuclease is a deoxyribonucleic acid (DNA).

42. The method of any one of claims 28-40, wherein the nucleic acid encoding said DNA endonuclease is a ribonucleic acid (RNA).

43. The method of claim 42, wherein the RNA encoding said DNA endonuclease is an mRNA.

44. The method of any one of claims 28-43, wherein the donor template is encoded in an AAV vector.

45. The method of any one of claims 28-44, wherein the donor template comprises a donor cassette comprising the nucleic acid sequence encoding the GOI or functional derivative, and wherein the donor cassette is flanked on one or both sides by a gRNA target site.

46. The method of claim 45, wherein the donor cassette is flanked on both sides by a gRNA target site.

47. The method of claim 45 or 46, wherein the gRNA target site is a target site for the gRNA of (a).

48. The method of claim 47, wherein the gRNA target site of the donor template is the reverse complement of a gRNA target site in the cell genome for the gRNA of (a).

49. The method of any one of claims 28-48, wherein said DNA endonuclease or nucleic acid encoding the DNA endonuclease is formulated in a liposome or lipid nanoparticle.

50. The method of claim 49, wherein said liposome or lipid nanoparticle also comprises the gRNA.

51. The method of any one of claims 28-50, comprising providing to the cell the DNA endonuclease precomplexed with the gRNA, forming an RNP complex.

52. The method of any one of claims 28-51, wherein the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell more than 4 days after the donor template of (c) is provided to the cell.

53. The method of any one of claims 28-52, wherein the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell at least 14 days after (c) is provided to the cell.

54. The method of claim 52 or 53, wherein one or more additional doses of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b).

55. The method of claim 54, wherein one or more additional doses of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) until a target level of targeted integration of the nucleic acid sequence encoding the GOI or functional derivative and/or a target level of expression of the nucleic acid sequence encoding the GOI or functional derivative is achieved.

56. The method of any one of claims 28-55, wherein the nucleic acid sequence encoding the GOI or functional derivative is expressed under the control of the endogenous albumin promoter.

57. The method of any one of claims 28-56, wherein said cell is a hepatocyte.

58. A genetically modified cell in which the genome of the cell is edited by the method of any one of claims 28-57.

59. The genetically modified cell of claim 58, wherein the nucleic acid sequence encoding the GOI or functional derivative is expressed under the control of the endogenous albumin promoter.

60. The genetically modified cell of claim 58 or 59, wherein the nucleic acid sequence encoding the GOI or functional derivative thereof is codon optimized for expression in the cell.

61. The genetically modified cell of any one of claims 58-60, wherein said cell is a hepatocyte.

62. Use of (a) to (c) in the manufacture of a medicament for treating a disorder or health condition in a subject, wherein:

(a) a gRNA comprising a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, 30, 18-20, 23-27, 29, 31-44, and 104, or nucleic acid encoding the gRNA;

(b) a DNA endonuclease or nucleic acid encoding the DNA endonuclease; and

(c) a donor template comprising a nucleic acid sequence encoding a gene-of-interest (GOI) or functional derivative.

63. The use of claim **Error! Reference source not found.**, wherein the gRNA comprises a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, and 30.

64. The use of claim 63, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 22.

65. The use of claim 63, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 21.

66. The use of claim 63, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 28.

67. The use of claim 63, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 30.

68. The use of any one of claims **Error! Reference source not found.**-67, wherein the GOI encodes a polypeptide selected from the group consisting of a therapeutic polypeptide and a prophylactic polypeptide.

69. The use of any one of claims **Error! Reference source not found.**-67, wherein the GOI encodes a protein selected from the group consisting of FVIII protein, FIX protein, alpha-1-antitrypsin, FXIII protein, FVII protein, FX protein, Protein C, serpin G1, or a functional derivative of any thereof.

70. The use of claim 69, wherein the GOI encodes a FVIII protein or a functional derivative of any thereof.

71. The use of claim 69, wherein the GOI encodes a FIX protein or a functional derivative of any thereof.

72. The use of claim 69, wherein the GOI encodes serpin G1 or a functional derivative of any thereof.

73. The use of any one of claims **Error! Reference source not found.**-72, wherein said subject is a patient having or suspected of having a disorder or health condition selected from the group consisting of hemophilia A, hemophilia B, MPS II, MPS1H, alpha-1-antitrypsin deficiency, FXIII deficiency, FVII deficiency, FX deficiency, Protein C deficiency, and HAE.

74. The use of claim 73, wherein the subject is a patient having or suspected of having hemophilia A.

75. The use of claim 73, wherein the subject is a patient having or suspected of having hemophilia B.

76. The use of claim 73, wherein the subject is a patient having or suspected of having HAE.

77. The use of any one of claims **Error! Reference source not found.**-72, wherein said subject is diagnosed with a risk of a disorder or health condition selected from the group

consisting of hemophilia A, hemophilia B, MPS II, MPS1H, alpha-1-antitrypsin deficiency, FXIII deficiency, FVII deficiency, FX deficiency, Protein C deficiency, and HAE.

78. The use of claim 77, wherein the subject is a patient is diagnosed with a risk of hemophilia A.

79. The use of claim 77, wherein the subject is a patient is diagnosed with a risk of hemophilia B.

80. The use of claim 77, wherein the subject is a patient is diagnosed with a risk of HAE.

81. The use of any one of claims 62-80, wherein said DNA endonuclease recognizes a protospacer adjacent motif (PAM) having the sequence NGG or NNGG, wherein N is any nucleotide; or a functional derivative thereof.

82. The use of any one of claims **Error! Reference source not found.**-81, wherein said DNA endonuclease is a type II Cas endonuclease or a functional derivative thereof.

83. The use of any one of claims **Error! Reference source not found.**-82, wherein said DNA endonuclease is Cas9.

84. The use of any one of claims **Error! Reference source not found.**-83, wherein the nucleic acid encoding said DNA endonuclease is codon optimized for expression in the cell.

85. The use of any one of claims **Error! Reference source not found.**-84, wherein the nucleic acid sequence encoding the GOI or functional derivative thereof is codon optimized for expression in the cell.

86. The use of any one of claims **Error! Reference source not found.**-85, wherein the nucleic acid encoding said DNA endonuclease is a deoxyribonucleic acid (DNA).

87. The use of any one of claims **Error! Reference source not found.**-85, wherein the nucleic acid encoding said DNA endonuclease is a ribonucleic acid (RNA).

88. The use of claim 87, wherein the RNA encoding said DNA endonuclease is an mRNA.

89. The use of any one of claims **Error! Reference source not found.**-88, wherein one or more of the gRNA of (a), the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b), and the donor template of (c) are formulated in a liposome or lipid nanoparticle.

90. The use of any one of claims **Error! Reference source not found.**-89, wherein the donor template is encoded in an AAV vector.

91. The use of any one of claims **Error! Reference source not found.**-90, wherein the donor template comprises a donor cassette comprising the nucleic acid sequence encoding the GOI or functional derivative, and wherein the donor cassette is flanked on one or both sides by a gRNA target site.

92. The use of claim 91, wherein the donor cassette is flanked on both sides by a gRNA target site.

93. The use of claim 91 or 92, wherein the gRNA target site is a target site for the gRNA of (a).

94. The use of claim 93, wherein the gRNA target site of the donor template is the reverse complement of the gRNA target site in the cell genome for the gRNA of (a).

95. The use of any one of claims **Error! Reference source not found.**-94, wherein providing the donor template to the cell comprises administering the donor template to the subject.

96. The use of claim 95, wherein the administration is via intravenous route.

97. The use of any one of claims **Error! Reference source not found.**-96, wherein said DNA endonuclease or nucleic acid encoding the DNA endonuclease is formulated in a liposome or lipid nanoparticle.

98. The use of claim 97, wherein said liposome or lipid nanoparticle also comprises the gRNA.

99. The use of claim 98, wherein providing the gRNA and the DNA endonuclease or nucleic acid encoding the DNA endonuclease to the cell comprises administering the liposome or lipid nanoparticle to the subject.

100. The use of claim 99, wherein the administration is via intravenous route.

101. The use of any one of claims **Error! Reference source not found.**-100, comprising providing to the cell the DNA endonuclease pre-complexed with the gRNA, forming an RNP complex.

102. The use of any one of claims **Error! Reference source not found.**-101, wherein the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell more than 4 days after the donor template of (c) is provided to the cell.

103. The use of any one of claims **Error! Reference source not found.**-102, wherein the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell at least 14 days after the donor template of (c) is provided to the cell.

104. The use of claim 102 or 103, wherein one or more additional doses of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b).

105. The use of claim 104, wherein one or more additional doses of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) until a target level of targeted integration of the nucleic acid sequence encoding the GOI or functional derivative and/or a target level of expression of the nucleic acid sequence encoding the GOI protein or functional derivative is achieved.

106. The use of any one of claims 102-105, wherein providing the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) to the cell comprises administering to the subject a lipid nanoparticle comprising nucleic acid encoding the DNA endonuclease and the gRNA.

107. The use of any one of claims 102-106, wherein providing the donor template of (c) to the cell comprises administering to the subject the donor template encoded in an AAV vector.

108. The use of any one of claims **Error! Reference source not found.**-107, wherein the nucleic acid sequence encoding the GOI or functional derivative is expressed under the control of the endogenous albumin promoter.

109. The use of any one of claims **Error! Reference source not found.**-108, wherein said cell is a hepatocyte.

110. The use of any one of claims **Error! Reference source not found.**-109, wherein the nucleic acid sequence encoding the GOI or functional derivative is expressed in the liver of the subject.

111. Use of A method of treating hemophilia A, hemophilia B, or hereditary angioedema in a subject comprising:

the administration of the genetically modified cell of any one of claims 58-61 to the subject.

112. The use of claim 111, wherein said genetically modified cell is autologous to the subject.

113. The use of claim 111 or 112 further comprising:

obtaining a biological sample from the subject wherein the biological sample comprises a hepatocyte cell, wherein the genetically modified cell is prepared from the hepatocyte.

114. A kit comprising one or more elements of the system of any one of claims 1-27, and further comprising instructions for use.