Claims

1. A modified FIX polypeptide, comprising an amino acid replacement in an unmodified FIX polypeptide at residue R318 or at a residue corresponding to 318, wherein:

the amino acid replacement is selected from among Y, E, F and W , or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide:

corresponding amino acid residues are identified by alignment of the unmodified FIX polypeptide with the polypeptide of SEQ ID NO:3;the unmodified FIX polypeptide comprises a sequence of amino acids set forth in any of SEQ ID NOS: 2, 3, 20 or 325, or a sequence that is an active fragment of a FIX polypeptide set forth in any SEQ ID NOS: 2, 3, 20 or 325, or a sequence having at least 90% sequence identity with the FIX polypeptide set forth in any of SEQ ID NOS: 2, 3, 20 or 325 or fragment thereof,

the modified FIX polypeptide exhibits at least one FIX activity or property that is improved compared to the unmodified FIX polypeptide that does not contain the modification; and

the activity is one or both of increased resistance to the inhibitory effects of AT-III and increased procoagulant activity.

2. The modified FIX polypeptide of claim 1 that comprises an amino acid replacement R318Y.

3. The modified FIX polypeptide of claim 1 or claim 2 that further comprises an amino acid replacement at R338 or at a residue corresponding to 338 in an unmodified FIX polypeptide.

4. The modified FIX polypeptide of any of claims 1-3 that comprises an amino acid replacement R338D or R338E or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

5. The modified FIX polypeptide of any of claims 1-4 that comprises an amino acid replacement R338E.

6. The modified FIX polypeptide of any of claims 1-5 comprising the amino acid replacements R318Y/R338E or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

7. The modified FIX polypeptide of any of claims 1-6 that further comprises an amino acid replacement at T343 or at an amino acid residue corresponding to amino acid residue T343 in an unmodified FIX polypeptide.

8. The modified FIX polypeptide of any of claims 1-7 that comprises an amino acid replacement T343R, T343E or T343D or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

9. The modified FIX polypeptide of any of claims 1-8 that comprises an amino acid replacement T343R.

10. The modified FIX polypeptide of claim 9 that comprises the amino acid replacements R318Y/R338E/T343R.

11. The modified FIX polypeptide of any of claims 1-9 that comprises amino acid replacements at residues R318 and T343 or at amino acid residues corresponding to amino acid residues R318 andT343 in an unmodified FIX polypeptide.

12. The modified FIX polypeptide of any of claims 1-9, comprising the amino acid replacements R318Y/T343R or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

13. The modified FIX polypeptide of any of claims 1-12, further comprising an amino acid replacement at E410 or at an amino acid residue corresponding to amino acid residue E410 in an unmodified FIX polypeptide, wherein the replacement is N or S.

14. The modified FIX polypeptide of any of claims 1-13 that comprises amino acid replacements R318Y/E410N or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

15. The modified FIX polypeptide of claim 1 or claim 2 that comprises an amino acid replacement R318Y and an amino acid replacement at an amino acid residue selected from among residues 338, 343, 403 and 410 of a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3 or at amino acid residues corresponding to residues 338, 343, 403 or 410 in an unmodified FIX polypeptide.

16. The modified FIX polypeptide of any of claims 1-15, comprising an amino acid replacement selected from among R338E, T343R, R403E and E410N in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3 or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

17. The modified FIX polypeptide of any of claims 1-16 that comprises amino acid replacements selected from among replacements R318Y/R338E/R403E/E410N, R318Y/R338E/T343R/R403E/E410N, R318Y/R338E/T343R/E410N, Y155F/R318Y/R338E/T343R/R403E, Y155F/K228N/K247N/N249S/R318Y/R338E/T343R/R403E/E410N, Y155F/K247N/N249S/R318Y/R338E/T343R/R403E, K247N/N249S/R318Y/R338E/T343R/R403E, R318Y/R338E/T343R, Y155F/K247N/N249S/R318Y/R338E/T343R, K228N/R318Y/R338E/T343R/R403E/E410N, K228N/K247N/N249S/R318Y/R338E/T343R/R403E, R318Y/R338E/T343R/R403E/E410S, Y155F/K247N/N249S/R318Y/R338E, K247N/N249S/R318Y/R338E/T343R, R318Y/T343R/E410N, Y155F/R318Y/R338E/R403E, R318Y/R338E/R403E, R318Y/R338E/E410N, K228N/R318Y/E410N, R318Y/R403E/E410N, D203N/F205T/R318Y/E410N, A103N/N105S/R318Y/R338E/R403E/E410N, D104N/K106S/R318Y/R338E/R403E/E410N, K228N/R318Y/R338E/R403E/E410N, I251S/R318Y/R338E/R403E/E410N, D104N/K106S/I251S/R318Y/R338E/R403E/E410N, D104N/K106S/R318Y/R338E/E410N, I251S/R318Y/E410N/R338E, D104N/K106S/I251S/R318Y/R338E/E410N, A103N/N105S/K247N/N249S/R318Y/R338E/R403E/E410N, D104N/K106S/K247N/N249S/R318Y/R338E/R403E/E410N, K228N/K247N/N249S/R318Y/R338E/R403E/E410N, A103N/N105S/Y155F/R318Y/R338E/R403E/E410N, D104N/K106S/Y155F/R318Y/R338E/R403E/E410N, Y155F/K228N/R318Y/R338E/R403E/E410N, Y155F/I251S/R318Y/R338E/R403E/E410N, K247N/N249S/R318Y/R338E/R403E/E410N, Y155F/R318Y/R338E/R403E/E410N, K247N/N249S/R318Y/R338E/E410N, Y155F/R318Y/R338E/E410N, Y155F/K247N/N249S/R318Y/R338E/E410N, R318Y/R338E/R403E/E410S, R318Y/R338E/R403E/E410N/T412V, R318Y/R338E/R403E/E410N/T412A, R318Y/R338E/R403E/T412A, R318Y/R338E/E410S, R318Y/R338E/T412A, R318Y/R338E/E410N/T412V, D85N/K228N/R318Y/R338E/R403E/E410N, N260S/R318Y/R338E/R403E/E410N, R318Y/R338E/N346D/R403E/E410N,

Y155F/R318Y/R338E/N346D/R403E/E410N,
K247N/N249S/N260S/R318Y/R338E/R403E/E410N,
D104N/K106S/N260S/R318Y/R338E/R403E/E410N,
Y155F/N260S/R318Y/R338E/R403E/E410N, Y155F/R318Y/R338E/T343R/R403E/E410N,
D104N/K106S/Y155F/K247N/N249S/R318Y/R338E/R403E/E410N,
D104N/K106S/K228N/K247N/N249S/R318Y/R338E/R403E/E410N,
Y155F/K228N/K247N/N249S/R318Y/R338E/R403E/E410N,
Y155F/K247N/N249S/N260S/R318Y/R338E/R403E/E410N,
D104N/K106S/R318Y/R338E/T343R/R403E/E410N, R318Y/R338E/N346Y/R403E/E410N,
R318Y/R338E/T343R/N346Y/R403E/E410N, R318Y/R338E/T343R/N346D/R403E/E410N,
R318Y/R338E/Y345A/R403E/E410N, K228N/I251S/R318Y/R338E/R403E/E410N,
R318Y/R338E/Y345A/N346D/R403E/E410N,
Y155F/K247N/N249S/R318Y/R338E/R403E, K247N/N249S/R318Y/R338E/R403E,
Y155F/K247N/N249S/R318Y/R403E/E410N, K247N/N249S/R318Y/R403E/E410N,
R318Y/R338E/T343R/R403E, Y155F/R318Y/R338E/T343R/E410N,
R318Y/T343R/R403E/E410N, Y155F/R318Y/T343R/R403E/E410N,
Y155F/K247N/N249S/R318Y/R338E/T343R/R403E/E410N,
K247N/N249S/R318Y/R338E/T343R/R403E/E410N,
Y155F/K228N/I251S/R318Y/R338E/R403E/E410N,
N260S/R318Y/R338E/T343R/R403E/E410N,
Y155F/N260S/R318Y/R338E/T343R/R403E/E410N,
K228N/K247N/N249S/R318Y/R338E/T343R/R403E/E410N,
Y155F/K247N/N249S/R318Y/R403E, Y155F/K247N/N249S/R318Y/E410N,
K247N/N249S/R318Y/R338E/T343R/E410N,
Y155F/K247N/N249S/R318Y/R338E/T343R/E410N,
Y155F/K247N/N249S/R318Y/T343R/R403E/E410N,
K247N/N249S/R318Y/T343R/R403E/E410N, Y155F/K247N/N249S/R318Y/T343R/R403E,
K247N/N249S/R318Y/T343R/R403E, Y155F/K247N/N249S/R318Y/T343R/E410N,
K247N/N249S/R318Y/T343R/E410N, Y155F/R318Y/R338E/T343R,
Y155F/R318Y/T343R/R403E, Y155F/R318Y/T343R/E410N,
K228N/K247N/N249S/R318Y/R338E/T343R/E410N,
Y155F/K247N/N249S/R318Y/T343R, Y155F/K247N/N249S/R318Y/R338E/R403E/E410N,
K228N/K247N/N249S/R318Y/T343R/R403E/E410N and Y155F/R318Y/R403E/E410N, or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

18. The modified FIX polypeptide of any of claims 1-17 that comprises amino acid replacements R318Y/R338E/R403E/E410N, R318Y/R338E/T343R/R403E/E410N, R318Y/R338E/T343R/E410N, Y155F/R318Y/R338E/T343R/R403E,
Y155F/K228N/K247N/N249S/R318Y/R338E/T343R/R403E/E410N,
Y155F/K247N/N249S/R318Y/R338E/T343R/R403E,
K247N/N249S/R318Y/R338E/T343R/R403E, R318Y/R338E/T343R,
Y155F/K247N/N249S/R318Y/R338E/T343R, K228N/R318Y/R338E/T343R/R403E/E410N,
K228N/K247N/N249S/R318Y/R338E/T343R/R403E, R318Y/R338E/T343R/R403E/E410S,
Y155F/K247N/N249S/R318Y/R338E, K247N/N249S/R318Y/R338E/T343R,
R318Y/T343R/E410N or Y155F/R318Y/R338E/R403E.

19. The modified FIX polypeptide of claim 18 that comprises amino acid replacements R318Y/R338E/R403E/E410N, R318Y/R338E/T343R/R403E/E410N, R318Y/R338E/T343R/E410N or R318Y/R338E/T343R.

20. The modified FIX polypeptide of claim 1 or 2, comprising an amino acid replacement at an amino acid residue selected from among D203, F205 and K228, or at an amino acid residue corresponding to amino acid residue D203, F205 or K228 in an unmodified FIX polypeptide.

21. The modified FIX polypeptide of any of claims 1-20, comprising an amino acid replacement selected from among D203N, F205T and K228N, or the same amino acid replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

22. The modified FIX polypeptide of any of claims 1-21, comprising the amino acid replacements D203N/F205T/R318Y or K228N/R318Y, or the same replacements at a corresponding amino acid residue in an unmodified FIX polypeptide.

23. The modified FIX polypeptide of any of claims 1-22 that comprises an amino acid replacement at amino acid position Y155 or at a residue corresponding to 155 that is selected from F or L.

24. The modified FIX polypeptide of claim 23 that comprises the amino acid replacement Y155F or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

25. The modified FIX polypeptide of any of claims 1-24, wherein the unmodified FIX polypeptide comprises a sequence of amino acids set forth in any of SEQ ID NOS: 2, 3, 20 and 325;

the polypeptides of SEQ ID NOS:3 and 20 are mature FIX polypeptides, and the polypeptides of SEQ ID NOs. 2 and 325 are precursor FIX polypeptides;

the residue numbers are determined by alignment with the unmodified mature FIX polypeptides whose sequences are set forth in SEQ ID NO:3 and 20; and

26. The modified FIX polypeptide of any of claims 1-24, wherein the unmodified FIX polypeptide consists a sequence of amino acids set forth in any of SEQ ID NOS: 2, 3, 20 and 325;

the polypeptides of SEQ ID NOS:3 and 20 are mature FIX polypeptides, and the polypeptides of SEQ ID NOs. 2 and 325 are precursor FIX polypeptides;

the residue numbers are determined by alignment with the unmodified mature FIX polypeptides whose sequences are set forth in SEQ ID NO:3 and 20.

27. The modified FIX polypeptide of claim 1, wherein the sequence of amino acid residues comprises the sequence set forth in any of SEQ ID NOS: 267, 330, 331, 334, 336, 345-348, 353-360, 366, 368, 369, 372-375, 378-381, 383-387, 393-395, 397, 398, 403-408, 410 , 411, 413 and 414.

28. The modified FIX polypeptide of claim 1, wherein the sequence of amino acid residues comprises the sequence set forth in any of SEQ ID NOS: 267, 330, 331, 334, 336, 345-348, 353, 354, 357-360, 366, 368, 369, 378, 379, 393, 394, 397, 398 and 405-407.

29. The modified FIX polypeptide of claim 1, wherein the modified FIX polypeptide comprises the sequence of amino acid residues set forth in SEQ ID NO:394.

30. The modified FIX polypeptide of claim 1, wherein the sequence of amino acid residues consists of the sequence set forth in any of SEQ ID NOS: 267, 330, 331, 334, 336, 345-348, 353-360, 366, 368, 369, 372-375, 378-381, 383-387, 393-395, 397, 398, 403-408, 410 , 411, 413 and 414.

31. The modified FIX polypeptide of claim 1, wherein the sequence of amino acid residues consists of the sequence set forth in any of SEQ ID NOS: 267, 330, 331, 334, 336, 345-348, 353, 354, 357-360, 366, 368, 369, 378, 379, 393, 394, 397, 398 and 405-407.

32. The modified FIX polypeptide of claim 1, wherein the modified FIX polypeptide consists of the sequence of amino acid residues set forth in SEQ ID NO:394.

33. The modified FIX polypeptide of any of claims 1-32, comprising one or more modifications that introduces and/or eliminates one or more glycosylation sites compared to the unmodified FIX polypeptide.

34. The modified FIX polypeptide of claim 33, wherein the glycosylation sites are selected from among, N-, O- and S-glycosylation sites.

35. The modified FIX polypeptide of claim 33 or claim 34, wherein 1, 2, 3, 4, 5, 6, 7, 8 or more glycosylation sites are introduced.

36. The modified FIX polypeptide of any of claims 1-33 that exhibits increased resistance to antithrombin III and/or heparin compared with the unmodified FIX polypeptide.

37. The modified FIX polypeptide of any of claims 1-36 that exhibits increased catalytic activity compared with the unmodified FIX polypeptide.

38. The modified FIX polypeptide of claim 37, wherein the increased catalytic activity is in the presence of FVIIIa.

39. The modified FIX polypeptide of claim 38, wherein the increased catalytic activity is in the absence of FVIIIa.

88. The modified FIX polypeptide of any of claims 1-87, wherein the unmodified FIX polypeptide comprises a sequence of amino acids set forth in any of SEQ ID NOS: 2, 3, 20 or 325, or a sequence having at least 95% sequence identity with the FIX polypeptide set forth in any of SEQ ID NOS: 2, 3, 20 or 325, or a sequence that is an active fragment of a FIX polypeptide set forth in any SEQ ID NOS: 2, 3, 20 or 325 ,wherein the modified FIX polypeptide has increased coagulant activity and/or increased resistance to ATIII-inhibition.

40. The modified FIX polypeptide of any of claims 1-88, wherein the unmodified FIX polypeptides comprises a sequence having at least 98% or 99% sequence identity with the FIX polypeptide set forth in any of SEQ ID NOS: 2, 3, 20 or 325 ,wherein the modified FIX polypeptide has increased coagulant activity and/or increased resistance to ATIII-inhibition.

41. The modified FIX polypeptide of any of claims 1-40, wherein the unmodified FIX polypeptide comprises a sequence of amino acids set forth in SEQ ID NO:2, 3, 20 or 325.

42. The modified FIX polypeptide of any of claims 1-41, wherein the unmodified FIX polypeptide consists of a sequence of amino acids set forth in SEQ ID NO:2, 3, 20 or 325 .

43. The modified FIX polypeptide of any of claims 1-42 that is a mature polypeptide.

44. The modified FIX polypeptide of any of claims 1-43 that is a two-chain polypeptide.

45. The modified FIX polypeptide of any of claims 1-44 that is a single-chain polypeptide.

46. The modified FIX polypeptide of any of claims 1-45 that is active or activated.

47. The modified FIX polypeptide of any of claims 1-46, wherein only the primary sequence is modified.

48. The modified FIX polypeptide of any of claims 1-47, further comprising a chemical modification or a post-translational modification.

49. The modified FIX polypeptide of claim 48, wherein the modified FIX polypeptide is glycosylated, carboxylated, hydroxylated, sulfated, phosphorylated, albuminated, or conjugated to a polyethylene glycol (PEG) moiety.

50. The modified FIX polypeptide of any of claims 1-49 that is a chimeric or fusion protein.

51. The modified FIX polypeptide of any of claims 1-50, comprising up to 1, 2, 3, 4, 5, 6, 7, 8, 9 or10 modifications, wherein:

the unmodified FIX polypeptide consists of a sequence of amino acids set forth in any of SEQ ID NOS: 2, 3, 20 or 325; and

the modified FIX polypeptide, when in active form, has increased coagulant activity and/or increased resistance to ATIII-inhibition.

52. A nucleic acid molecule, comprising a sequence of nucleotides encoding a modified FIX polypeptide of any of claims 1-51.

53. A vector, comprising the nucleic acid molecule of claim 52.

54. The vector of claim 53, wherein the vector is a prokaryotic vector, viral vector, or a eukaryotic vector.

55. The vector of claim 53 or claim 54, wherein the vector is a mammalian vector.

56. The vector of claim 54 or claim 55, wherein the vector is a viral vector selected from among an adenovirus, an adeno-associated-virus, a retrovirus, a herpes virus, a lentivirus, a poxvirus, and a cytomegalovirus.

57. A cell, comprising the vector of any of claims 53-56.

58. The cell of claim 57 that is a eukaryotic cell.

59. The cell of claim 58, wherein the eukaryotic cell is a mammalian cell.

60. The cell of claim 59, wherein the mammalian cell is selected from among baby hamster kidney cells (BHK-21) or 293 cells or CHO cells.

61. A method of producing a modified FIX polypeptide , comprising:

culturing a cell of any of claims 57-60, whereby the cell expresses the modified FIX polypeptide; and

optionally isolating the modified FIX polypeptide..

62. A pharmaceutical composition, comprising a modified FIX polypeptide of any of claims 1-51 in a pharmaceutically acceptable vehicle.

63. The pharmaceutical composition of claim 62 that is formulated for local, systemic, or topical administration.

64. The pharmaceutical composition of claim 62 that is formulated for oral, nasal, pulmonary, buccal, transdermal, subcutaneous, intraduodenal, enteral, parenteral, intravenous, or intramuscular administration.

65. The pharmaceutical composition of any of claims 62-64 that is formulated for controlled-release.

66. The pharmaceutical composition of any of claims 62-65 that is formulated for

single-dosage administration.

67. A modified FIX polypeptide of any of claims 1-51 for use in treating a disease or condition that is treated by administration of FIX or a pro-coagulant.

68. Use of a modified FIX polypeptide of any of claims 1-51 in the preparation of a medicament for treatment of a disease or condition that is treated by administration of FIX or a pro-coagulant.

69. The modified FIX polypeptide of claim 67 or the use of claim 68, wherein the disease or condition is treated by administration of active FIX (FIXa).

70. The modified FIX polypeptide of claim 67 or the use of claim 68, wherein treatment ameliorates or alleviates the symptoms associated with the disease or condition.

71. The modified FIX polypeptide or the use of any of claims 67-70, wherein the disease or condition to be treated is selected from among blood coagulation disorders, hematologic disorders, hemorrhagic disorders, hemophilias, and bleeding disorders.

72. The modified FIX polypeptide or the use of claim 71, wherein the disease or condition is hemophilia B.

Claims

1. A modified FIX polypeptide, comprising an amino acid replacement in an unmodified FIX polypeptide, wherein the amino acid replacement is selected from among replacement of tyrosine (Y) at amino acid residue R318 (R318Y), R318E, R318F, R318W, R318D, R318I, R318K, R318L, R318M, R318S, R318V, S61A, S61C, S61D, S61E, S61F, S61G, S61I, S61K, S61L, S61P, S61R, S61V, S61W, S61Y, D64A, D64C, D64F, D64H, D64I, D64L, D64M, D64P, D64R, D64S, D64T, D64W, Y155F, Y155L, N157D, N157E, N157F, N157I, N157K, N157L, N157M, N157R, N157V, N157W, N157Y, S158A, S158D, S158E, S158F, S158G, S158I, S158K, S158L, S158M, S158R, S158V, S158W, S158Y, N167D, N167Q, N167E, N167F, N167G, N167H, N167I, N167K, N167L, N167M, N167P, N167R, N167V, N167W, N167Y, T169A, T169D, T169E, T169F, T169G, T169I, T169K, T169L, T169M, T169P, T169R, T169S, T169V, T169W, T169Y, T172A, T172D, T172E, T172F, T172G, T172I, T172K, T172L, T172M, T172P, T172R, T172S, T172V, T172W, T172Y, D203M, D203Y, D203F, D203H, D203I, D203K, D203L, D203R, D203V, D203W, A204M, A204Y, A204F, A204I, A204W, E239S, E239R, E239K, E239D, E239F, E239I, E239L, E239M, E239T, E239V, E239W, E239Y, H257F, H257E, H257D, H257I, H257K, H257L, H257M, H257Q, H257R, H257V, H257W, R312Y, R312L, R312C, R312D, R312E, R312F, R312I, R312K, R312M, R312P, R312S, R312T, R312V, R312W, K316M, K316D, K316F, K316H, K316I, K316L, K316R, K316V, K316W, K316Y, F342L, F342Y, T343R, T343E, T343D, T343F, T343I, T343K, T343L, T343M, T343S, T343V, T343W, T343Y, N346Y, N346E, N346F, N346H, N346I, N346K, N346L, N346M, N346Q, N346R, N346V, N346W,K400E, K400C, K400D, K400F, K400G, K400L, K400M, K400P, K400S, K400T, K400V, K400Y, R403D, R403F, R403I, R403K, R403L, R403M, R403S, R403V, R403Y, E410D, E410S, E410A, E410F,

E410G, E410I, E410K, E410L, E410M, E410P, E410R, E410T, E410V, E410W, E410Y, T412A, T412V, T412C, T412D, T412E, T412F, T412G, T412I, T412M, T412P, T412W and T412Y in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3, or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide, wherein:
corresponding amino acid residues are identified by alignment of the unmodified FIX polypeptide with the polypeptide of SEQ ID NO:3;
the modified FIX polypeptide does not contain the modifications F342I/T343R/Y345T;
the modified FIX polypeptide exhibits at least one FIX activity or property that is improved compared to the unmodified FIX polypeptide that does not contain the modification; and
the activity or property is selected from among one or more of increased resistance to the inhibitory effects of AT-III, the inhibitory effects of AT-III/heparin, the inhibitory effects of heparin and increased procoagulant activity.

2. The modified FIX polypeptide of claim 1, comprising an amino acid replacement selected from among amino acid replacements R318Y, R318E, R318F, R318W, S61A, D64A, Y155F, Y155L, N157D, S158A, S158D, S158E, N167D, N167Q, T169A, T172A, D203M, D203Y, A204M, A204Y, E239S, E239R, E239K, H257F, H257E, R312Y, R312L, K316M, T343R, T343E, T343D, N346Y, K400E, R403D, E410D, E410S, E410A, T412A and T412V, or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

3. The modified FIX polypeptide of claim 1 or claim 2 that comprises an amino acid replacement at R318 or at a residue corresponding to 318, wherein the amino acid replacement is selected from among Y, E, F and W.

4. The modified FIX polypeptide of claim 3 that comprises an amino acid replacement R318Y.

5. The modified FIX polypeptide of claim 3 or claim 4 that further comprises an amino acid replacement at R338 or at a residue corresponding to 338 in an unmodified FIX polypeptide.

6. The modified FIX polypeptide of any of claims 3-5 that comprises an amino acid replacement R338D or R338E or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

7. The modified FIX polypeptide of any of claims 3-6 that comprises an amino acid replacement R338E.

8. The modified FIX polypeptide of any of claims 3-6 that comprises amino acid replacements at residues R318 and R338 or at amino acid residues corresponding to amino acid residues R318 and R338 in an unmodified FIX polypeptide.

9. The modified FIX polypeptide of any of claims 3-8 comprising the amino acid replacements R318Y/R338E or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

10. The modified FIX polypeptide of claim 3 or claim 4 that further comprises an amino acid replacement at T343 or at an amino acid residue corresponding to amino acid residue T343 in an unmodified FIX polypeptide.

11. The modified FIX polypeptide of any of claims 1-10 that comprises an amino acid replacement T343R, T343E or T343D or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

12. The modified FIX polypeptide of any of claims 1-11 that comprises an amino acid replacement T343R.

13. The modified FIX polypeptide of claim 12 that comprises the amino acid replacements R318Y/R338E/T343R.

14. The modified FIX polypeptide of any of claims 3-13 that comprises amino acid replacements at residues R318 and T343 or at amino acid residues corresponding to amino acid residues R318 andT343 in an unmodified FIX polypeptide.

15. The modified FIX polypeptide of any of claims 3-14 comprising the amino acid replacements R318Y/T343R or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

16. The modified FIX polypeptide of any of claims 3-15, further comprising an amino acid replacement at E410 or at an amino acid residue corresponding to amino acid residue E410 in an unmodified FIX polypeptide, wherein the replacement is N or S.

17. The modified FIX polypeptide of any of claims 3-16 that comprises amino acid replacements R318Y/E410N or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

18. The modified FIX polypeptide of any of claims 1-17 that comprises an amino acid replacement R318Y and an amino acid replacement at an amino acid residue selected from among residues 338, 343, 403 and 410 of a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3 or at amino acid residues corresponding to residues 338, 343, 403 or 410 in an unmodified FIX polypeptide.

19. The modified FIX polypeptide of any of claims 1-18, comprising an amino acid replacement selected from among R338E, T343R, R403E and E410N in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3 or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

20. The modified FIX polypeptide of any of claims 1-19 that comprises amino acid replacements selected from among replacements R318Y/R338E/R403E/E410N, R318Y/R338E/T343R/R403E/E410N, R318Y/R338E/T343R/E410N, Y155F/R318Y/R338E/T343R/R403E, Y155F/K228N/K247N/N249S/R318Y/R338E/T343R/R403E/E410N, Y155F/K247N/N249S/R318Y/R338E/T343R/R403E, K247N/N249S/R318Y/R338E/T343R/R403E, R318Y/R338E/T343R, Y155F/K247N/N249S/R318Y/R338E/T343R, K228N/R318Y/R338E/T343R/R403E/E410N, K228N/K247N/N249S/R318Y/R338E/T343R/R403E, R318Y/R338E/T343R/R403E/E410S,

Y155F/K247N/N249S/R318Y/R338E,
K247N/N249S/R318Y/R338E/T343R, R318Y/T343R/E410N,
Y155F/R318Y/R338E/R403E, R318Y/R338E/R403E,
R318Y/R338E/E410N, K228N/R318Y/E410N, R318Y/R403E/E410N,
D203N/F205T/R318Y/E410N,
A103N/N105S/R318Y/R338E/R403E/E410N,
D104N/K106S/R318Y/R338E/R403E/E410N,
K228N/R318Y/R338E/R403E/E410N,
I251S/R318Y/R338E/R403E/E410N,
D104N/K106S/I251S/R318Y/R338E/R403E/E410N,
D104N/K106S/R318Y/R338E/E410N, I251S/R318Y/E410N/R338E,
D104N/K106S/I251S/R318Y/R338E/E410N,
A103N/N105S/K247N/N249S/R318Y/R338E/R403E/E410N,
D104N/K106S/K247N/N249S/R318Y/R338E/R403E/E410N,
K228N/K247N/N249S/R318Y/R338E/R403E/E410N,
A103N/N105S/Y155F/R318Y/R338E/R403E/E410N,
D104N/K106S/Y155F/R318Y/R338E/R403E/E410N,
Y155F/K228N/R318Y/R338E/R403E/E410N,
Y155F/I251S/R318Y/R338E/R403E/E410N,
K247N/N249S/R318Y/R338E/R403E/E410N,
Y155F/R318Y/R338E/R403E/E410N,
K247N/N249S/R318Y/R338E/E410N, Y155F/R318Y/R338E/E410N,
Y155F/K247N/N249S/R318Y/R338E/E410N,
R318Y/R338E/R403E/E410S, R318Y/R338E/R403E/E410N/T412V,
R318Y/R338E/R403E/E410N/T412A, R318Y/R338E/R403E/T412A,
R318Y/R338E/E410S, R318Y/R338E/T412A,
R318Y/R338E/E410N/T412V, D85N/K228N/R318Y/R338E/R403E/E410N,
N260S/R318Y/R338E/R403E/E410N,
R318Y/R338E/N346D/R403E/E410N,
Y155F/R318Y/R338E/N346D/R403E/E410N,
K247N/N249S/N260S/R318Y/R338E/R403E/E410N,
D104N/K106S/N260S/R318Y/R338E/R403E/E410N,

Y155F/N260S/R318Y/R338E/R403E/E410N,
Y155F/R318Y/R338E/T343R/R403E/E410N,
D104N/K106S/Y155F/K247N/N249S/R318Y/R338E/R403E/E410N,
D104N/K106S/K228N/K247N/N249S/R318Y/R338E/R403E/E410N,
Y155F/K228N/K247N/N249S/R318Y/R338E/R403E/E410N,
Y155F/K247N/N249S/N260S/R318Y/R338E/R403E/E410N,
D104N/K106S/R318Y/R338E/T343R/R403E/E410N,
R318Y/R338E/N346Y/R403E/E410N,
R318Y/R338E/T343R/N346Y/R403E/E410N,
R318Y/R338E/T343R/N346D/R403E/E410N,
R318Y/R338E/Y345A/R403E/E410N,
K228N/I251S/R318Y/R338E/R403E/E410N,
R318Y/R338E/Y345A/N346D/R403E/E410N,
Y155F/K247N/N249S/R318Y/R338E/R403E,
K247N/N249S/R318Y/R338E/R403E,
Y155F/K247N/N249S/R318Y/R403E/E410N,
K247N/N249S/R318Y/R403E/E410N, R318Y/R338E/T343R/R403E,
Y155F/R318Y/R338E/T343R/E410N, R318Y/T343R/R403E/E410N,
Y155F/R318Y/T343R/R403E/E410N,
Y155F/K247N/N249S/R318Y/R338E/T343R/R403E/E410N,
K247N/N249S/R318Y/R338E/T343R/R403E/E410N,
Y155F/K228N/I251S/R318Y/R338E/R403E/E410N,
N260S/R318Y/R338E/T343R/R403E/E410N,
Y155F/N260S/R318Y/R338E/T343R/R403E/E410N,
K228N/K247N/N249S/R318Y/R338E/T343R/R403E/E410N,
Y155F/K247N/N249S/R318Y/R403E,
Y155F/K247N/N249S/R318Y/E410N,
K247N/N249S/R318Y/R338E/T343R/E410N,
Y155F/K247N/N249S/R318Y/R338E/T343R/E410N,
Y155F/K247N/N249S/R318Y/T343R/R403E/E410N,
K247N/N249S/R318Y/T343R/R403E/E410N,
Y155F/K247N/N249S/R318Y/T343R/R403E,

K247N/N249S/R318Y/T343R/R403E,
Y155F/K247N/N249S/R318Y/T343R/E410N,
K247N/N249S/R318Y/T343R/E410N, Y155F/R318Y/R338E/T343R,
Y155F/R318Y/T343R/R403E, Y155F/R318Y/T343R/E410N,
K228N/K247N/N249S/R318Y/R338E/T343R/E410N,
Y155F/K247N/N249S/R318Y/T343R,
Y155F/K247N/N249S/R318Y/R338E/R403E/E410N,
K228N/K247N/N249S/R318Y/T343R/R403E/E410N and
Y155F/R318Y/R403E/E410N, or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

21. The modified FIX polypeptide of any of claims 1-20 that comprises amino acid replacements R318Y/R338E/R403E/E410N, R318Y/R338E/T343R/R403E/E410N, R318Y/R338E/T343R/E410N, Y155F/R318Y/R338E/T343R/R403E,
Y155F/K228N/K247N/N249S/R318Y/R338E/T343R/R403E/E410N,
Y155F/K247N/N249S/R318Y/R338E/T343R/R403E,
K247N/N249S/R318Y/R338E/T343R/R403E, R318Y/R338E/T343R,
Y155F/K247N/N249S/R318Y/R338E/T343R,
K228N/R318Y/R338E/T343R/R403E/E410N,
K228N/K247N/N249S/R318Y/R338E/T343R/R403E,
R318Y/R338E/T343R/R403E/E410S,
Y155F/K247N/N249S/R318Y/R338E,
K247N/N249S/R318Y/R338E/T343R, R318Y/T343R/E410N or
Y155F/R318Y/R338E/R403E.

22. The modified FIX polypeptide of any of claims 1-21 that comprises amino acid replacements R318Y/R338E/R403E/E410N, R318Y/R338E/T343R/R403E/E410N or R318Y/R338E/T343R/E410N.

23. The modified FIX polypeptide of claim 3 or 4, comprising an amino acid replacement at an amino acid residue selected from among D203, F205 and K228, or at an amino acid residue corresponding to amino acid residue D203, F205 or K228 in an unmodified FIX polypeptide.

24. The modified FIX polypeptide of any of claims 1-23, comprising an amino acid replacement selected from among D203N, F205T and K228N, or the same amino acid replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

25. The modified FIX polypeptide of any of claims 1-24, comprising the amino acid replacements D203N/F205T/R318Y or K228N/R318Y, or the same replacements at a corresponding amino acid residue in an unmodified FIX polypeptide.

26. The modified FIX polypeptide of any of claims 1-25 that comprises an amino acid replacement at amino acid position Y155 or at a residue corresponding to 155 that is selected from F or L.

27. The modified FIX polypeptide of any of claims 1-26 that comprises the amino acid replacement Y155F or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

28. The modified FIX polypeptide of claim 26 or claim 27 that further comprises an amino acid replacement at residue 103, 104, 105, 106, 228, 251, 260 or 346 of a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3, or

at an amino acid residue corresponding to 103, 104, 105, 106, 228, 251, 260 or 346 in an unmodified FIX polypeptide.

29. The modified FIX polypeptide of any of claims 1-28 that comprises an amino acid replacement selected from among A103N, D104N, N105S, K106S, K228N, I251S, N260S and N346D, or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

30. The modified FIX polypeptide of any of claims 1-29 that comprises amino acid replacements selected from among Y155F/K228N, Y155F/I251S, D104N/K106S/Y155F/K228N, Y155F/N346D, Y155F/N260S, Y155F/N260S/N346D, D104N/K106S/Y155F/N260S, D104N/K106S/Y155F/K228N, A103N/N105S/Y155F and D104N/K106S/Y155F, or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

31. The modified FIX polypeptide of claim 26 or claim 27 that further comprises an amino acid replacement at a residue selected from among 338, 403 and 410 of a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3, or at an amino acid residue corresponding to 338, 403 or 410 in an unmodified FIX polypeptide.

32. The modified FIX polypeptide of any of claims 1-31 that comprises an amino acid replacement selected from among R338E, R403E and E410N, or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

33. The modified FIX polypeptide of any of claims 1-32 that comprises amino acid replacements selected from among Y155F/R338E/T343R/R403E/E410N, Y155F/R338E/T343R/R403E, Y155F/R338E/T343R/R403E/E410S, Y155F/N260S/R338E/T343R/R403E, Y155F/T343R/R403E/E410N, Y155F/R338E/T343R/E410N and Y155F/R338E/R403E/E410N.

34. The modified FIX polypeptide of any of any of claims 1-33 that comprises the amino acid replacement Y155F and amino acid replacements at amino acid positions 247 and/or 249 of a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3.

35. The modified FIX polypeptide of any of claims 1-34 that comprises the amino acid replacement Y155F/K247N/N249S.

36. The modified FIX polypeptide of any of claims 1-35 that comprises amino acid replacements
Y155F/K247N/N249S/R338E/R403E/E410N,
D104N/K106S/Y155F/K228N/K247N/N249S,
D104N/K106S/Y155F/K247N/N249S, Y155F/K228N/K247N/N249S,
Y155F/K247N/N249S/N260S,
D104N/K106S/Y155F/K247N/N249S/N260S,
D104N/K106S/Y155F/K247N/N249S,
Y155F/K247N/N249S/T343R/R403E,
Y155F/K247N/N249S/R338E/T343R/R403E/E410N,
Y155F/K247N/N249S/R338E/R403E,
Y155F/K247N/N249S/R338E/T343R,
Y155F/K247N/N249S/R338E/E410N,
Y155F/K247N/N249S/R338E/T343R/R403E,
Y155F/K247N/N249S/R338E/T343R/E410N,
Y155F/K247N/N249S/T343R/R403E/E410N,

Y155F/K247N/N249S/T343R/E410N and Y155F/K247N/N249S/R403E/E410N, or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

37. The modified FIX polypeptide of any of claims 1-36 that comprises amino acid replacements Y155F/K247N/N249S/R338E/R403E/E410N.

38. The modified FIX polypeptide of any of claims 1-37 that comprises an amino acid replacement at T343 or at a residue corresponding to 343 that is selected from among R, E and D.

39. The modified FIX polypeptide of claim 38 that comprises an amino acid replacement T343R or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

40. The modified FIX polypeptide of claim 38 or claim 39 that comprises an amino acid replacement at an amino acid residue selected from among R338, Y345 and N346, or at the corresponding amino acid residue in an unmodified FIX polypeptide.

41. The modified FIX polypeptide of any of claims 1-40, comprising an amino acid replacement R338E, Y345T, N346Y or N346D, or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

42. The modified FIX polypeptide of any of claims 1-41, comprising amino acid replacements T343R/Y345T, T343R/N346D,

T343R/N346Y or R338E/T343R, or the same replacements at a corresponding amino acid residue in an unmodified FIX polypeptide.

43. The modified FIX polypeptide of any of claims 1-42 that comprises amino acid replacements K247N/N249S/R338E/T343R/R403E/E410N, R338E/T343R/E410N, Y155F/I251S/R338E/T343R/R403E, R338E/T343R/R403E/E410N, R338E/T343R/R403E, K247N/N249S/R338E/T343R/R403E or K247N/N249S/R338E/T343R/E410N.

44. The modified FIX polypeptide of any of claims 1-43 that comprises amino acid replacements K247N/N249S/R338E/T343R/R403E/E410N, R338E/T343R/E410N or Y155F/I251S/R338E/T343R/R403E.

45. The modified FIX polypeptide of claim 38 or claim 39, comprising an amino acid replacement at an amino acid residue selected from among K247, N249, R403 and E410, or at an amino acid residue corresponding to amino acid residue K247, N249, R403 or E410 in an unmodified FIX polypeptide.

46. The modified FIX polypeptide of any of claims 1-45, comprising an amino acid replacement selected from among K247N, N249S, R403E and E410N or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

47. The modified FIX polypeptide of any of claims 1-46, comprising the amino acid replacement K247N/N249S/T343R/R403E/E410N.

48. The modified FIX polypeptide of any of claims 1-47, comprising amino acid replacements at one or more positions corresponding to 1, 5, 53, 61, 64, 85, 103, 104, 105, 106, 108, 148, 155, 157, 158, 159, 167, 169, 172, 179, 202, 202, 203, 204, 205, 228, 239, 241, 243, 247, 249, 251, 257, 259, 260, 262, 265, 284, 293, 312, 314, 315, 316, 317, 318, 319, 320, 321, 333, 338, 343, 345, 346, 392, 394, 400, 403, 410, 412 and 413 of a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3, or to a corresponding position in an unmodified FIX polypeptide.

49. The modified FIX polypeptide of any of claims 1-48, comprising an amino acid replacement selected from among Y1N, K5A, S53A, S61A, D64A, D64N, D85N, A103N, D104N, N105S, N105T, K106N, K106S, K106T, V108S, V108T, T148A, Y155F, Y155H, N157D, N157Q, S158A, S158D, S158E, T159A, N167D, N167Q, T169A, T172A, T179A, V202M, V202Y, D203M, D203Y, D203N, A204M, A204Y, F205S, F205T, K228N, E239N, E239S, E239A, E239R, E239K, T241N, H243S, H243T, K247N, N249S, N249T, I251S, H257F, H257Y, H257E, H257S, N260S, A262S, A262T, Y284N, K293E, K293A, R312Q, R312A, R312Y, R312L, F314N, H315S, K316S, K316T, K316M, G317N, R318E, R318Y, R318N, R318A, S319N, A320S, L321N, L321S, L321T, R333A, R333E, R338A, R338E, F342I, T343R, T343E, T343Q, Y345A, Y345T, N346D, N346T, K392N, K394S, K394T, K400A, K400E, R403A, R403E, E410Q, E410S, E410N, E410A, E410D, T412V, T412A and K413N, or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

50. The modified FIX polypeptide of any of claims 1-49, comprising amino acid replacements selected from replacements R318E/R403E, E410N/T412A or E410N/T412V or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

51. A modified Factor IX (FIX) polypeptide, comprising two amino acid replacements in an unmodified FIX polypeptide, wherein:
the first amino acid replacement is at an amino acid residue selected from among 53, 61, 64, 85, 103, 104, 105, 106, 108, 155, 158, 159, 167, 169, 172, 179, 202, 203, 204, 205, 228, 239, 241, 243, 247, 249, 251, 257, 259, 260, 262, 265, 284, 293, 312, 314, 315, 316, 317, 318, 319, 321, 333, 338, 343, 345, 346, 392, 394, 400, 403, 410, 412 and 413 in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3; or a corresponding amino acid residue in an unmodified FIX polypeptide, wherein corresponding amino acid residues are identified by alignment of the unmodified FIX polypeptide with the polypeptide of SEQ ID NO:3; and
the second amino acid replacement is at an amino acid residue selected from among 5, 53, 61, 64, 85, 155, 158, 159, 167, 239, 260, 284, 293, 312, 318, 333, 338, 346, 400, 403, 410, 412 and 413 in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3, or a corresponding amino acid residue in an unmodified FIX polypeptide, wherein corresponding amino acid residues are identified by alignment of the unmodified FIX polypeptide with the polypeptide of SEQ ID NO:3.

52. The modified FIX polypeptide of claim 51, wherein the first amino acid replacement is replacement with an amino

acid residue selected from among alanine (Ala, A); arginine (Arg, R); asparagine (Asn, N); aspartic acid (Asp, D); cysteine (Cys, C); glutamic acid (Glu, E); glutamine (Gln, Q); glycine (Gly, G); histidine (His, H); isoleucine (Ile, I); leucine (Leu, L); lysine (Lys, K); methionine (Met, M); phenylalanine (Phe, F); proline (Pro, P); serine (Ser, S); threonine (Thr, T); tryptophan (Trp, W); tyrosine (Tyr, Y); and valine (Val, V); wherein
the replacing amino acid is not the same as the amino acid it replaces.

53. The modified FIX polypeptide claim 51 or claim 52, wherein the first amino acid replacement is replacement with an amino acid residue selected from among alanine (Ala, A); asparagine (Asn, N); aspartic acid (Asp, D), glutamic acid (Glu, E); glutamine (Gln, Q); histidine (His, H); isoleucine (Ile, I); leucine (Leu, L); lysine (Lys, K); methionine (Met, M); phenylalanine (Phe, F); serine (Ser, S); threonine (Thr, T); tyrosine (Tyr, Y); and valine (Val, V), or a conservative amino acid replacement.

54. The modified FIX polypeptide of any of claims 51-53, wherein the first amino acid replacement is selected from among S53A, S61A, D64A, D64N, D85N, A103N, D104N, N105S, K106S, K106N, V108S, Y155F, Y155H, Y155Q, S158A, S158D, S158E, T159A, N167D, N167Q, T169A, T172A, T179A, V202M, V202Y, D203M, D203Y, A204M, A204Y, K228N, E239A, E239N, E239S, E239R, E239K, T241N, H243S, K247N, N249S, I251S, H257F, H257E, H257F, H257Y, H257S, Y259S, N260S, A262S, K265T, Y284N, K293E, K293A, R312Q, R312A, R312Y, R312L, F314N, H315S, K316S, K316N, K316A, K316E, K316S, K316M, G317N, R318A, R318E, R318Y, R318N, S319N, A320S, L321S,

K321N, R333A, R333E, R333S, R338A, R338E, R338L, F342I, T343R, T343E, T343Q, Y345A, Y345T, N346D, N346Y, K392N, K394S, K400A, K400E, R403A, R403E, E410Q, E410N, E410D, E410S, E410A, T412A, T412V and K413N, or a conservative amino acid replacement or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

55. The modified FIX polypeptide of any of claims 51-52, wherein the second amino acid replacement is replacement with an amino acid residue selected from among alanine (Ala, A); arginine (Arg, R); asparagine (Asn, N); aspartic acid (Asp, D); cysteine (Cys, C); glutamic acid (Glu, E); glutamine (Gln, Q); glycine (Gly, G); histidine (His, H); isoleucine (Ile, I); leucine (Leu, L); lysine (Lys, K); methionine (Met, M); phenylalanine (Phe, F); proline (Pro, P); serine (Ser, S); threonine (Thr, T); tryptophan (Trp, W); tyrosine (Tyr, Y); and valine (Val, V); wherein the replacing amino acid is not the same as the amino acid it replaces.

56. The modified FIX polypeptide of any of claims 51-55, wherein the second amino acid replacement is replacement with an amino acid residue selected from among alanine (Ala, A); arginine (Arg, R); asparagine (Asn, N); aspartic acid (Asp, D); glutamic acid (Glu, E); glutamine (Gln, Q); histidine (His, H); leucine (Leu, L); lysine (Lys, K); phenylalanine (Phe, F); serine (Ser, S); threonine (Thr, T); tyrosine (Tyr, Y); and valine (Val, V), or a conservative amino acid replacement.

57. The modified FIX polypeptide of any of claims 51-56, wherein the second amino acid replacement is selected from among K5A, S53A, S61A, D64A, D64N, D85N, Y155F, Y155H, Y155Q, S158A, S158D, S158E, T159A, N167D, N167Q, E239A, E239N, E239S, E239R, E239K, N260S, Y284N, K293E, K293A, R312Q, R312A, R312Y, R312L, R318A, R318E, R318Y, R318N, R333A, R333E, R333S, R338A, R338E, R338L, N346D, N346Y, K400A, K400E, R403A, R403E, E410Q, E410N, E410D, E410S, E410A, T412A, T412V and K413N, or a conservative amino acid replacement or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

58. The modified FIX polypeptide of any of claims 51-57, wherein the first amino acid replacement is at a position selected from among 318, 155, 247, 249, 338, 403 and 410 or at a corresponding amino acid residue in an unmodified FIX polypeptide.

59. The modified FIX polypeptide of any of claims 51-58, wherein the first amino acid replacement is selected from among R318Y, Y155F, K247N, N249S, R338E, R403E and E410N or is the same amino acid replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

60. The modified FIX polypeptide of any of claims 51-59, wherein the second amino acid replacement is at a position selected from among 338, 155, 247, 249, 318, 403 and 410, or is at a corresponding amino acid residue in an unmodified FIX polypeptide.

61. The modified FIX polypeptide of any of claims 51-60, wherein the second amino acid replacement is selected from

among R338E, Y155F, K247N, N249S, R318Y, R403E and E410N or is the same replacement at a corresponding amino acid residue.

62. The modified FIX polypeptide of any of claims 51-61, comprising amino acid replacements selected from among amino acid replacements K400E/R403E, D85N/K228N, D85N/I251S, K400A/R403A, R338A/R403A, R338E/R403E, K293A/R403A, K293E/R403E, R318A/R403A, R338E/E410N, K228N/E410N, K228N/R338E, K228N/R338A and R403E/E410N, or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

63. The modified FIX polypeptide of any of claims 51-62, comprising a further amino acid replacement at a position selected from among 53, 61, 64, 85, 103, 104, 105, 106, 108, 155, 158, 159, 167, 169, 172, 179, 202, 203, 204, 205, 228, 239, 241, 243, 247, 249, 251, 257, 259, 260, 262, 265, 284, 293, 312, 314, 315, 316, 317, 318, 319, 321, 333, 338, 343, 346, 345, 392, 394, 400, 403, 410, 412 and 413 in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3.

64. The modified FIX polypeptide of claim 62 or claim 63, wherein a further amino acid replacement corresponds to an amino acid replacement selected from among Y1N, Y5A, S53A, S61A, D64A, D64N, D85N, A103N, D104N, N105S, K106S, K106N, V108S, Y155F, Y155H, Y155Q, S158A, S158D, S158E, T159A, N167D, N167Q, T169A, T172A, T179A, V202M, V202Y, D203M, D203Y, A204M, A204Y, K228N, E239A, E239N, E239S, E239R, E239K, T241N, H243S, K247N, N249S, I251S, H257F, H257E, H257Y, H257S, Y259S, N260S, A262S, K265T, Y284N, K293E, K293A, R312Q, R312A, R312Y, R312L, F314N, H315S, K316S,

K316N, K316A, K316E, K316S, K316M, G317N, R318A, R318E, R318Y, R318N, S319N, A320S, L321S, R333A, R333E, R333S, R338A, R338E, R338L, F342I, T343R, T343E, T343Q, Y345A, Y345T, N346D, N346Y, K392N, K394S, K400A, K400E, R403A, R403E, E410Q, E410N, E410D, E410S, E410A, T412A, T412V and K413N, or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

65. The modified FIX polypeptide of any of claims 51-64, comprising amino acid replacements selected from among amino acid replacements corresponding to replacements D85N/D203N/F205T, D85N/D104N/K106S/I251S, K293A/R338A/R403A, K293E/R338E/R403E, R338E/R403E/E410N, D203N/F205T/K228N, D203N/F205T/E410N, D203N/F205T/R338E, D203N/F205T/R338A, D203N/F205T/R338E/R403E, K228N/R338E/R403E, K247N/N249S/N260S, D104N/K106S/N260S, D104N/K106S/K228N/K247N/N249S, A103N/N105S/K228N, D104N/K106S/K228N, A103N/N105S/I251S, D104N/K106S/I251S, A103N/N105S/K247N/N249S, D104N/K106S/K247N/N249S, K228N/K247N/N249S, D104N/K106S/N260S, Y259F/K265T/Y345T, D104N/K106S/K247N/N249S/N260S and K247N/N249S/R338E/R403E/E410N.

66. The modified FIX polypeptide of any of claims 1-65, comprising one or more modifications that introduces and/or eliminates one or more glycosylation sites compared to the unmodified FIX polypeptide.

67. The modified FIX polypeptide of claim 66, wherein the glycosylation sites are selected from among, N-, O- and S-glycosylation sites.

68. The modified FIX polypeptide of claim 66 or claim 67, wherein 1, 2, 3, 4, 5, 6, 7, 8 or more glycosylation sites are introduced.

69. The modified FIX polypeptide of any of claims 1-66 that exhibits increased resistance to antithrombin III and/or heparin compared with the unmodified FIX polypeptide.

70. The modified FIX polypeptide of claim 69, wherein the modified FIX polypeptide exhibits at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more increased resistance to antithrombin III and/or heparin compared with the unmodified FIX polypeptide.

71. The modified FIX polypeptide of any of claims 1-70 that exhibits increased catalytic activity compared with the unmodified FIX polypeptide.

72. The modified FIX polypeptide of claim 71, wherein the increased catalytic activity is in the presence of FVIIIa.

73. The modified FIX polypeptide of claim 72, wherein the increased catalytic activity is in the absence of FVIIIa.

74. The modified FIX polypeptide of any of claims 69-73, wherein catalytic activity of the modified FIX polypeptide is at least about or is 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more greater than the catalytic of an unmodified FIX polypeptide.

75. The modified FIX polypeptide of any of claims 1-74 further exhibits improved pharmacokinetic properties compared with the unmodified FIX polypeptide.

76. The modified FIX polypeptide of claim 75, wherein the improved pharmacokinetic properties are selected from among one or more of decreased clearance, altered volume of distribution, increased *in vivo* recovery, increased total modified FIX polypeptide exposure *in vivo*, increased serum half-life, and increased mean resonance time (MRT) compared to the unmodified FIX polypeptide.

77. The modified FIX polypeptide of claim 76, wherein the improved pharmacokinetic property increased serum half-life, and the serum half life is α, β, or γ phase.

78. The modified FIX polypeptide of claim 76 or claim 77, wherein the improved pharmacokinetic property is decreased clearance, and clearance of the modified FIX polypeptide is decreased by at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of the clearance of an unmodified FIX polypeptide.

79. The modified FIX polypeptide of claim 76, wherein the improved pharmacokinetic property is altered volume of distribution, and the volume of distribution is increased or decreased.

80. The modified FIX polypeptide of claim 79, wherein the altered volume of distribution is decreased volume of

distribution, and the volume of distribution of the modified FIX polypeptide is decreased by at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of the volume of distribution of an unmodified FIX polypeptide.

81. The modified FIX polypeptide of claim 79, wherein the altered volume of distribution is increased volume of distribution, and the volume of distribution of the modified FIX polypeptide is increased by at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more of the volume of distribution of an unmodified FIX polypeptide.

82. The modified FIX polypeptide of claim 76, wherein the improved pharmacokinetic property is increased *in vivo* recovery, and the *in vivo* recovery of the modified FIX polypeptide is increased by at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more of the *in vivo* recovery of an unmodified FIX polypeptide.

83. The modified FIX polypeptide of claim 76, wherein the improved pharmacokinetic property is increased total modified FIX polypeptide exposure *in vivo*, and the total modified FIX polypeptide exposure *in vivo* is increased by at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more of the total exposure *in vivo* of an unmodified FIX polypeptide.

84. The modified FIX polypeptide of claim 76, wherein the improved pharmacokinetic property is increased serum half-life, and the serum half-life is increased by at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more of the serum half-life of an unmodified FIX polypeptide.

85. The modified FIX polypeptide of claim 76, wherein the improved pharmacokinetic property is increased MRT, and the MRT is increased by at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more of the MRT *in vivo* of an unmodified FIX polypeptide.

86. The modified FIX polypeptide of any of claims 1-85 that exhibits increased procoagulant activity compared with the unmodified FIX polypeptide.

87. The modified FIX polypeptide of claim 86, wherein the procoagulant activity of the modified FIX polypeptide is at least about or is 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more greater than the procoagulant activity of an unmodified FIX polypeptide.

88. The modified FIX polypeptide of any of claims 1-87, wherein the unmodified FIX polypeptide comprises a sequence of amino acids set forth in any of SEQ ID NOS: 2, 3, 20 or 325, or a sequence having at least 60% sequence identity with the FIX polypeptide set forth in any of SEQ ID NOS: 2, 3, 20 or 325, or a sequence that is an active fragment of a FIX polypeptide set forth in any SEQ ID NOS: 2, 3, 20 or 325.

89. The modified FIX polypeptide of any of claims 1-88, wherein the unmodified FIX polypeptides comprises a sequence having at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with the FIX polypeptide set forth in any of SEQ ID NOS: 2, 3, 20 or 325.

90. The modified FIX polypeptide of any of claims 1-89, wherein the unmodified FIX polypeptide has a sequence of amino acids set forth in SEQ ID NO:2, 3, 20 or 325.

91. A modified FIX polypeptide, comprising a sequence of amino acids set forth in any of SEQ ID NOS: 76, 84-87, 90-91, 95-97, 100, 102, 104-105, 113, 121-122, 125-126, 129-134, 138, 146-173, 175-176, 178-185, 188, 193-194, 196-198, 200, 202-206, 208-210, 212, 215-272, 326-41 and 413-417, or a sequence of amino acids that exhibits at least 60% amino acid sequence identity with a sequence of amino acids set forth in any of SEQ ID NOS: 76, 84-87, 90-91, 95-97, 100, 102, 104-105, 113, 121-122, 125-126, 129-134, 138, 146-173, 175-176, 178-185, 188, 193-194, 196-198, 200, 202-206, 208-210, 212, 215-272, 326-41 and 413-417.

92. The modified FIX polypeptide of claim 91, comprising a sequence of amino acids that has at least or about 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the modified FIX polypeptide set forth in any of SEQ ID NOS: 76, 84-87, 90-91, 95-97, 100, 102, 104-105, 113, 121-122, 125-126, 129-134, 138, 146-173, 175-176, 178-185, 188, 193-194, 196-198, 200, 202-206, 208-210, 212, 215-272, 326-41 and 413-417.

93. The modified FIX polypeptide of claim 91, wherein the modified FIX polypeptide comprises a sequence of amino acids set forth in any of SEQ ID NOS: 76, 84-87, 90-91, 95-97, 100, 102, 104-105, 113, 121-122, 125-126, 129-134, 138, 146-173, 175-176, 178-185, 188, 193-194, 196-198, 200, 202-206, 208-210, 212, 215-272, 326-41 and 413-417.

94. The modified FIX polypeptide of any of claims 1-93 that is a human polypeptide.

95. The modified FIX polypeptide of any of claims 1-94 that is a non-human polypeptide.

96. The modified FIX polypeptide of any of claims 1-95 that is a mature polypeptide.

97. The modified FIX polypeptide of any of claims 1-96 that is a two-chain polypeptide.

98. The modified FIX polypeptide of any of claims 1-97 that is a single-chain polypeptide.

99. The modified FIX polypeptide of any of claims 1-98 that is active or activated.

100. The modified FIX polypeptide of claim 99, wherein activation is effected by proteolytic cleavage by Factor XI (FXIa) or FVIIa.

101. The modified FIX polypeptide of claim 99, wherein activation is effected by proteolytic cleavage by the Tissue Factor/Factor VIIa complex.

102. The modified FIX polypeptide of any of claims 1-101, wherein only the primary sequence is modified.

103. The modified FIX polypeptide of any of claims 1-101, further comprising a chemical modification or a post-translational modification.

104. The modified FIX polypeptide of claim 103, wherein the modified FIX polypeptide is glycosylated, carboxylated, hydroxylated, sulfated, phosphorylated, albuminated, or conjugated to a polyethylene glycol (PEG) moiety.

105. The modified FIX polypeptide of any of claims 1-104 that is a chimeric or fusion protein.

106. The modified FIX polypeptide of any of claims 1-105, comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more modifications.

107. (Amended) The modified FIX polypeptide of any of claims 1-106, comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 or 30 amino acid replacements.

108. The modified FIX polypeptide of any of claims 1-107, wherein the one or more properties are selected from among Factor VIIIa binding, Factor X binding, phospholipid binding, and/or coagulant activity of a FIX polypeptide.

109. A nucleic acid molecule, comprising a sequence of nucleotides encoding a modified FIX polypeptide of any of claims 1-108.

110. A vector, comprising the nucleic acid molecule of claim 109.

111. The vector of claim 109, wherein the vector is a prokaryotic vector, viral vector, or a eukaryotic vector.

112. The vector of claim 109 or claim 110, wherein the vector is a mammalian vector.

113. The vector of claim 109 or claim 110, wherein the viral vector is selected from among an adenovirus, an adeno-associated-virus, a retrovirus, a herpes virus, a lentivirus, a poxvirus, and a cytomegalovirus.

114. A cell, comprising the vector of any of claims 109-112.

115. The cell of claim 114, that is a eukaryotic cell.

116. The cell of claim 115, wherein the eukaryotic cell is a mammalian cell.

117. The cell of claim 116, wherein the mammalian cell is selected from among baby hamster kidney cells (BHK-21) or 293 cells or CHO cells.

118. The cell of any of claims claim 114-117, wherein the cell expresses the modified FIX polypeptide.

119. A pharmaceutical composition, comprising a modified FIX polypeptide of any of claims 1-109 in a pharmaceutically acceptable vehicle.

120. The pharmaceutical composition of claim 119 that is formulated for local, systemic, or topical administration.

121. The pharmaceutical composition of claim 119 that is formulated for oral, nasal, pulmonary, buccal, transdermal, subcutaneous, intraduodenal, enteral, parenteral, intravenous, or intramuscular administration.

122. The pharmaceutical composition of any of claims 119-121 that is formulated for controlled-release.

123. The pharmaceutical composition of any of claims 119-122 that is formulated for single-dosage administration.

124. A method, comprising treating a subject by administering the pharmaceutical composition of any of claims 119-123, wherein the subject has a disease or condition that is treated by administration of FIX or a procoagulant.

125. The method of claim 124, wherein the disease or condition is treated by administration of active FIX (FIXa).

126. The method of claim 124 or claim 125, wherein treatment with the pharmaceutical composition ameliorates or alleviates the symptoms associated with the disease or condition.

127. The method of any of claims 128-130, further comprising monitoring the subject for changes in the symptoms associated with disease or condition that is treated by administration of FIX or a procoagulant.

128 The method of any of claims 124-127, wherein the disease or condition to be treated is selected from among blood coagulation disorders, hematologic disorders, hemorrhagic disorders, hemophilias and bleeding disorders.

129. The method of claim 128, wherein the hemophilia is hemophilia B.

130. The method of any of claims 128-133, further comprising administering one or more additional coagulation factors.

131. The method of claim 130, wherein the one or more additional coagulation factors are selected from among plasma purified or recombinant coagulation factors, procoagulants, such as vitamin K, vitamin K derivative and protein C inhibitors, plasma, platelets, red blood cells and corticosteroids.

132. A kit, comprising the pharmaceutical composition of any of claims 119-123, a device for administration of the composition and, optionally, instructions for administration.

133. A modified FIX polypeptide of any of claims 1-109 for use in treating a disease or condition that is treated by administration of FIX or a pro-coagulant.

134. Use of a pharmaceutical composition of any of claims 123-127 in the preparation of a medicament for treatment of a disease or condition that is treated by administration of FIX or a pro-coagulant.

135. The modified FIX polypeptide of claim 133 or the use of claim 134, wherein the disease or condition is treated by administration of active FIX (FIXa).

136. The modified FIX polypeptide of claim 132 or the use of claim 134, wherein treatment ameliorates or alleviates the symptoms associated with the disease or condition.

137. The modified FIX polypeptide of any of claims 133, 135 and 136, or the use of any of claims 134-136, wherein the disease or condition to be treated is selected from among blood coagulation disorders, hematologic disorders, hemorrhagic disorders, hemophilias, and bleeding disorders.

138. The modified FIX polypeptide of any of claims 133, 135 and 136, or the use of any of claims 134-136, wherein the hemophilia is hemophilia B.