CLAIMS

WHAT IS CLAIMED IS:

1. A recombinant factor VIII fusion protein comprising a factor VIII polypeptide and at least a first extended recombinant polypeptide (XTEN), wherein said factor VIII polypeptide comprises an A1 domain including an a1 acidic spacer region, an A2 domain including an a2 acidic spacer region, an A3 domain including an a3 acidic spacer region, a C1 domain, a C2 domain and optionally all or a portion of a B domain, and wherein said first XTEN is linked to said factor VIII polypeptide at (i) the C-terminus of said factor VIII polypeptide; (ii) within the B domain of said factor VIII polypeptide if all or a portion of the B domain is present; (iii) within the A1 domain of said factor VIII polypeptide; (iv) within the A2 domain of said factor VIII polypeptide; (v) within the A3 domain of said factor VIII polypeptide; (vi) within the C1 domain of said factor VIII polypeptide; (vii) within the C2 domain of said factor VIII polypeptide; (viii) at the N-terminus of said factor VIII polypeptide, (ix) between two domains of said factor VIII polypeptide; and wherein when compared to a corresponding factor VIII protein not linked to XTEN, the fusion protein (a) retains at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% 100%, 200%, 300%, 400%, or 500% of the procoagulant activity in an in vitro coagulation assay, or (b) exhibits reduced binding to an anti-factor VIII antibody in an *in vitro* binding assay.

2. The recombinant factor VIII fusion protein of claim 1, comprising a factor VIII polypeptide and at least a first extended recombinant polypeptide (XTEN), wherein said factor VIII polypeptide comprises an A1 domain including an a1 acidic spacer region, an A2 domain including an a2 acidic spacer region, an A3 domain including an a3 acidic spacer region, a C1 domain, a C2 domain and optionally all or a portion of a B domain, and wherein said first XTEN is linked to said factor VIII polypeptide at (i) the C-terminus of said factor VIII polypeptide; (ii) within the B domain of said factor VIII polypeptide if all or a portion of the B domain is present; (iii) within the A1 domain of said factor VIII polypeptide; (iv) within the A2 domain of said factor VIII polypeptide; (v) within the A3 domain of said factor VIII polypeptide; (vi) within the C1 domain of said factor VIII polypeptide; (vii) within the C2 domain of said factor VIII polypeptide; or (viii) between two domains of said factor VIII polypeptide; and when compared to a corresponding factor VIII protein not linked to XTEN, the fusion protein (a) retains at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% 100%, 200%, 300%, 400%, or 500% of the procoagulant activity in an in vitro coagulation assay, and (b) exhibits reduced binding to an anti-factor VIII antibody in an *in vitro* binding assay.

3. The recombinant factor VIII fusion protein of claim 1 or 2, wherein the first XTEN is linked to said factor VIII polypeptide at an insertion site selected from Table 5, Table 6, Table 7, Table 8, or Table 9.

4. A recombinant factor VIII fusion protein comprising a factor VIII polypeptide and at least a first extended recombinant polypeptide (XTEN), wherein said factor VIII polypeptide comprises an A1 domain including an a1 acidic spacer region, an A2 domain including an a2 acidic spacer region, an A3 domain including an a3 acidic spacer region, a C1 domain, a C2 domain and optionally all or a

portion of a B domain, and wherein said first XTEN is linked to said factor VIII polypeptide at an insertion site selected from Table 6 and Table 7 and wherein the fusion protein retains at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%,100%, 200%, 300%, 400%, or 500% of the procoagulant activity, when measured by an in vitro coagulation assay, compared to a corresponding factor VIII protein not linked to XTEN.

5. The recombinant factor VIII fusion protein of any of claims 1-4, wherein the factor VIII polypeptide has at least about 80%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99%, or about 100% sequence identity to a sequence selected from the group consisting of the sequences in Table 1, the sequence depicted in FIG. 3, and the sequence depicted in FIG. 4, when optimally aligned.

6. The recombinant factor VIII fusion protein of any one of claims 1-5, comprising at least a second XTEN linked to said factor VIII polypeptide at the C-terminus of said factor VIII polypeptide or within or optionally replacing the B domain of said factor VIII polypeptide.

7. The recombinant factor VIII fusion protein of any one of claims 1-5, comprising at least a second XTEN linked to said factor VIII polypeptide within or optionally replacing the B domain of said factor VIII polypeptide.

8. The recombinant factor VIII fusion protein of any one of claims 1-7, wherein the factor VIII polypeptide comprises a B-domain deleted variant of human factor VIII, wherein the B-domain deletion starts from a first position at about amino acid residue number 741 to about 750 and ending at a second position at about amino acid residue number 1635 to about 1648 with reference to full-length human factor VIII sequence as set forth in FIG. 3.

9. The recombinant factor VIII fusion protein of any one of claims 6-8, wherein the first XTEN sequence is linked to said factor VIII polypeptide at the C-terminus of said factor VIII polypeptide, and the second XTEN is linked to said factor VIII polypeptide within the B domain of said factor VIII polypeptide, wherein the second XTEN is linked to the C-terminal end of about amino acid residue number 741 to about 750 and to the N-terminal end of amino acid residue numbers 1635 to about 1648 with reference to full-length human factor VIII sequence as set forth in FIG. 3, wherein the cumulative length of all XTENs is at least about 84 amino acid residues.

10. The recombinant factor VIII fusion protein of any one of claims 1-9, wherein the fusion protein has at least about 80%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99%, or at least about 100% sequence identity compared to a sequence of comparable length selected from the group consisting of the sequences of Table 21, when optimally aligned.

11. The recombinant factor VIII fusion protein of any one of claims 1-10, comprising at least a second XTEN, optionally a third XTEN, optionally a fourth XTEN, optionally a fifth XTEN and

optionally a sixth XTEN, wherein each of the second, third, fourth, fifth, or sixth XTEN is linked to said factor VIII polypeptide at a second, third, fourth, fifth, or sixth site selected from the group consisting of:

(a) an insertion site from Table 5, Table 6, Table 7, Table 8, or Table 9;

(b) an insertion site within 6 amino acids of amino acid residue number 32, 220, 224, 336, 339, 390, 399, 416, 603, 1656, 1711, 1725, 1905 or 1910 with reference to full-length human factor VIII sequence as set forth in FIG. 3;

(c) an insertion site between any two adjacent domains of said factor VIII polypeptide, wherein said two adjacent domains are selected from the group consisting of A1 and A2 domains, A2 and B domains, B and A3 domains, A3 and C1 domains, and C1 and C2 domains;

(d) within the B domain of said factor VIII polypeptide, wherein the second XTEN is linked to the C-terminal end of about amino acid residue number 741 to about 750 and to the N-terminal end of amino acid residue numbers 1635 to about 1648 with reference to full-length human factor VIII sequence as set forth in FIG. 3;

(e) the C-terminus of said factor VIII polypeptide; and

(f) the N-terminus of said factor VIII polypeptide.

12. The recombinant factor VIII fusion protein of claim 11, wherein the first XTEN is separated from the second XTEN by at least 10 amino acids, at least 50 amino acids, at least 100 amino acids, at least 200 amino acids, at least 300 amino acids, or at least 400 amino acids.

13. The recombinant factor VIII fusion protein of any one of claims 1-12, wherein said fusion protein exhibits a terminal half-life at least about 3 hours, or at least about 4 hours, or at least about 6 hours, or at least about 12 hours, or at least about 13 hours, or at least about 14 hours, or at least about 16 hours, or at least about 24 hours, or at least about 48 hours, or at least about 72 hours, or at least about 96 hours, or at least about 120 hours, or at least about 144 hours, or at least about 7 days, or at least about 14 days, or at least about 21 days when administered to a subject, wherein said subject is selected from human and factor VIII/von Willebrand factor double knock-out mouse.

14. The recombinant factor VIII fusion protein of any one of claims 1-13, wherein each XTEN has at least about 80%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99%, or about 100% sequence identity compared to an XTEN of comparable length selected from the group consisting of the sequences in Table 4, Table 13, Table 14, Table 15, Table 16, and Table 17, when optimally aligned.

15. The recombinant factor VIII fusion protein of any one of claims 1-14, wherein said fusion protein exhibits reduced binding of the fusion protein to an anti-factor VIII antibody and greater procoagulant activity as compared to a corresponding factor VIII polypeptide not linked to an XTEN.

16. The recombinant factor VIII fusion protein of claim 15, wherein the fusion protein has at least 30%, 40%, 50%, 80%, 100%, 200%, 300%, 400%, or 500% greater procoagulant activity in the presence of the anti-FVIII antibody compared to a corresponding factor VIII polypeptide not linked to an XTEN, when the procoagulant activity is assayed by an in vitro coagulation assay.

17. The recombinant factor VIII fusion protein of claim 15, wherein the reduced binding of the fusion protein to the anti-factor VIII antibody compared to a corresponding factor VIII polypeptide not linked to an XTEN is determined using a Bethesda assay using anti-factor VIII antibody selected from the group consisting of the antibodies in Table 10 and a polyclonal antibody from a hemophilia A patient with factor VIII inhibitors.

18. The recombinant factor VIII fusion protein of claim 17, wherein the reduced binding to anti-factor VIII antibody and the retained procoagulant activity of the fusion protein is evidenced by a lower Bethesda titer of at least about 2, 4, 6, 8, 10, 12, 15, 20, 30, 40, 50, 60, 70, 80, 100, or 200 Bethesda units for the fusion protein compared to that for the factor VIII polypeptide not linked to XTEN.

19. A recombinant factor VIII fusion protein comprising at least one extended recombinant polypeptide (XTEN), said fusion protein having a structure of formula IX:

$(A1_N)$-$(S)_a$-$(XTEN)_t$-$(S)_b$-$(A1_C)$-$(A2_N)$-$(S)_c$-$(XTEN)_u$-$(S)_d$-$(A2_C)$-(B_N)-$(S)_e$-$(XTEN)_v$-$(S)_f$-(B_C)-$(A3_N)$-$(S)_g$-$(XTEN)_w$-$(S)_h$-$(A3_C)$-$(C1_N)$-$(S)_i$-$(XTEN)_x$-$(S)_j$-$(C1_C)$-$(C2_N)$-$(S)_k$-$(XTEN)_y$-$(S)_l$-$(C2_C)$-$(S)_m$-$(XTEN)_z$

IX, wherein independently for each occurrence,

a) $A1_N$ is a fragment of the A1 domain of factor VIII having a sequence ranging from at least amino acid residue number 1, with reference to full-length human factor VIII sequence as set forth in FIG. 3, to no more than amino acid residue number 371;

b) $A1_c$ is a fragment of the A1 domain of factor VIII having a sequence ranging from at least amino acid residue number 2 to no more than amino acid residue number 372, with the priviso that no sequence of the $A1_N$ fragment is duplicated in the $A1_c$ is a fragment;

c) $A2_N$ is a fragment of the A2 domain of factor VIII having a sequence ranging from at least amino acid residue number 373 to no more than amino acid residue number 739;

d) $A2_c$ is a fragment of the A2 domain of factor VIII having a sequence ranging from at least amino acid residue number 374 to no more than amino acid residue number 740, with the priviso that no sequence of the $A2_N$ fragment is duplicated in the $A2_c$ is a fragment;

e) B_N is a fragment of the B domain of factor VIII having a sequence ranging from at least amino acid residue number 741 to no more than amino acid residue number 1647;

f) B_c is a fragment of the B domain of factor VIII having a sequence ranging from at least amino acid residue number 742 to no more than amino acid residue number 1648, with the priviso that no sequence of the B_N fragment is duplicated in the B_c is a fragment;

g) $A3_N$ is a fragment of the A3 domain of factor VIII having a sequence ranging from at least amino acid residue number 1649 to no more than amino acid residue number 2018;

h) $A3_c$ is a fragment of the A3 domain of factor VIII having a sequence ranging from at least amino acid residue number 1650 to no more than amino acid residue number 2019, with the priviso that no sequence of the $A3_N$ fragment is duplicated in the $A3_c$ is a fragment;

i) $C1_N$ is a fragment of the C1 domain of factor VIII having a sequence ranging from at least amino acid residue number 2020 to no more than amino acid residue number 2171;

j) $C1_c$ is a fragment of the C1 domain of factor VIII having a sequence ranging from at least amino acid residue number 2021 to no more than amino acid residue number 2172, with the priviso that no sequence of the $C1_N$ fragment is duplicated in the $C1_c$ is a fragment;

k) $C2_N$ is a fragment of the C2 domain of factor VIII having a sequence ranging from at least amino acid residue number 2173 to no more than amino acid residue number 2331;

l) $C2_c$ is a fragment of the C2 domain of factor VIII having a sequence ranging from at least amino acid residue number 2174 to no more than amino acid residue number 2332, with the priviso that no sequence of the $C2_N$ fragment is duplicated in the $C2_c$ is a fragment;

m) S is a spacer sequence having between 1 to about 50 amino acid residues that can optionally include a cleavage sequence or amino acids compatible with restrictions sites;

n) a is either 0 or 1;

o) b is either 0 or 1;

p) c is either 0 or 1;

q) d is either 0 or 1;

r) e is either 0 or 1;

s) f is either 0 or 1;

t) g is either 0 or 1;

u) h is either 0 or 1;

v) i is either 0 or 1;

w) j is either 0 or 1;

x) k is either 0 or 1;

y) l is either 0 or 1;

z) m is either 0 or 1;

aa) t is either 0 or 1;

bb) u is either 0 or 1;

cc) v is either 0 or 1;

dd) w is either 0 or 1;

ee) x is either 0 or 1;

ff) y is either 0 or 1;

gg) z is either 0 or 1 with the proviso that $t + u + v + w + x + y + z \geq 1$;

hh) each XTEN is independently an extended recombinant protein;

and wherein the fusion protein has at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% of the procoagulant activity of a corresponding factor VIII polypeptide not linked to an XTEN or wherein said fusion protein exhibits reduced binding to an anti-factor VIII antibody as compared to the corresponding factor VIII not linked to XTEN when measured by an in vitro assay which is an ELISA or Bethesda assay.

20. The recombinant factor VIII fusion protein of claim 19, wherein z is 1.

21. The recombinant factor VIII fusion protein of claim 19, wherein v is 1 and wherein $(XTEN)_v$ is linked to the C-terminal end of about amino acid residue number 741 to about 750 and to the N-terminal end of amino acid residue numbers 1635 to about 1648 with reference to full-length human factor VIII sequence as set forth in FIG. 3.

22. The recombinant factor VIII fusion protein of claim 19, wherein the sum of t, u, v, w, x, y, and z equals 2, 3, 4, 5, or 6.

23. The recombinant factor VIII fusion protein of claim 22, wherein the sum of t, u, v, w, x, y, and z equals 2, and v is 1 and z is 1.

24. The recombinant factor VIII fusion protein of claim 22, wherein the sum of t, u, v, w, x, y, and z equals 3, v and z each equal 1, and one of t, u, w, x or y is 1.

25. The recombinant factor VIII fusion protein of claim 22, wherein the sum of t, u, v, w, x, y, and z equals 4, v and w and z each equal 1, and one of t, u, x or y is 1.

26. The recombinant factor VIII fusion protein of any one of the preceding claims, wherein the cumulative length of all XTENs is between about 84 to about 3000, or between about 144 to about 2000, or about 288 to about 1000 amino acid residues.

27. The recombinant factor VIII fusion protein of any one of claims 19 to 26, wherein each XTEN has at least about 80%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99%, or about 100% sequence identity compared to an XTEN of comparable length selected from the group consisting of the sequences in Table 4, Table 13, Table 14, Table 15, Table 16, and Table 17, when optimally aligned.

28. The recombinant factor VIII fusion protein of any one of claims 19 to 27, wherein each XTEN is independently linked to said fusion protein at insertion sites selected from Table 5, Table 6, Table 7, Table 8, and Table 9.

29. The recombinant factor VIII fusion protein of any one of claims 19 to 28, wherein an XTEN is inserted immediately downstream of an amino acid which corresponds to an amino acid in mature native human factor VIII selected from the group consisting of amino acid residue numbers 32, 220, 224, 336, 339, 399, 416, 603, 1656, 1711, 1725, 1905 and 1910.

30. A recombinant factor VIII fusion protein comprising: a first polypeptide comprising formula X: (A1) – a1 – (A2) – a2 – [B]; and a second polypeptide comprising Formula XI: a3 – (A3) – (C1) - (C2);

wherein the first polypeptide and the second polypeptide are fused or exist as a heterodimer;

wherein, a) A1 is an A1 domain of factor VIII; b) A2 is an A2 domain of factor VIII; c) [B] is a B domain of factor VIII, a fragment thereof, or is deleted; d) A3 is an A3 domain of factor VIII; e) C1 is a C1 domain of factor VIII; f) C2 is a C2 domain of factor VIII; g) a1, a2, and a3 are acidic spacer regions;

wherein the A1 domain comprises an XTEN permissive loop-1 (A1-1) region and an XTEN permissive loop-2 (A1-2) region;

wherein the A2 domain comprises an XTEN permissive loop-1 (A2-1) region and an XTEN permissive loop-2 (A2-2) region;

wherein the A3 domain comprises an XTEN permissive loop-1 (A3-1) region and an XTEN permissive loop-2 (A3-2) region;

wherein an XTEN sequence is inserted into at least one of the regions A1-1, A1-2, A2-1, A2-2, A3-1, or A3-2; and

wherein the recombinant factor VIII protein exhibits procoagulant activity.

31. A recombinant factor VIII fusion protein comprising: a first polypeptide comprising formula X: (A1) – a1 – (A2) – a2 – [B]; and a second polypeptide comprising formula XI: a3 – (A3) – (C1) - (C2);

wherein the first polypeptide and the second polypeptide are fused or exist as a heterodimer;

wherein, a) A1 is an A1 domain of factor VIII; b) A2 is an A2 domain of factor VIII; c) [B] is a B domain of factor VIII, a fragment thereof, or is deleted or optionally not present; d) A3 is an A3 domain of factor VIII; e) C1 is a C1 domain of factor VIII; f) C2 is a C2 domain of factor VIII; g) a1, a2, and a3 are acidic spacer regions;

wherein an XTEN is inserted into a3; and

wherein the fusion protein exhibits procoagulant activity.

32. The recombinant factor VIII fusion protein of claim 30 or claim 31, wherein the first polypeptide and the second polypeptide form a single polypeptide chain comprising the formula (A1) – a1 – (A2) – a2 – [B] – [a3] – (A3) – (C1) – (C2).

33. The recombinant factor VIII fusion protein of claim 30, wherein the XTEN permissive loops are contained within surface-exposed, flexible loop structures, and wherein A1-1 is located between beta strand 1 and beta strand 2, A1-2 is located between beta strand 11 and beta strand 12, A2-1 is located between beta strand 22 and beta strand 23, A2-2 is located between beta strand 32 and beta strand 33, A3-1 is located between beta strand 38 and beta strand 39 and A3-2 is located between beta strand 45 and beta strand 46, according to the secondary structure of mature Factor VIII stored as Accession Number 2R7E of the DSSP database.

34. The recombinant factor VIII fusion protein of claim 33, wherein the surface-exposed, flexible loop structure comprising A1-1 corresponds to a region in native mature human factor VIII from about amino acid 15 to about amino acid 45.

35. The recombinant factor VIII fusion protein of claim 33, wherein A1-1 corresponds to a region in native mature human factor VIII from about amino acid 18 to about amino acid 41.

36. The recombinant factor VIII fusion protein of claim 33, wherein the surface-exposed, flexible loop structure comprising A1-2 corresponds to a region in native mature human factor VIII from about amino acid 201 to about amino acid 232.

37. The recombinant factor VIII fusion protein of claim 33, wherein A1-2 corresponds to a region in native mature human factor VIII from about amino acid 218 to about amino acid 229.

38. The recombinant factor VIII fusion protein of claim 33, wherein the surface-exposed, flexible loop structure comprising A2-1 corresponds to a region in native mature human factor VIII from about amino acid 395 to about amino acid 421.

39. The recombinant factor VIII fusion protein of claim 33, wherein A2-1 corresponds to a region in native mature human factor VIII from about amino acid 397 to about amino acid 418.

40. The recombinant factor VIII fusion protein of claim 33, wherein the surface-exposed, flexible loop structure comprising A2-2 corresponds to a region in native mature human factor VIII from about amino acid 577 to about amino acid 635.

41. The recombinant factor VIII fusion protein of claim 33, wherein A2-2 corresponds to a region in native mature human factor VIII from about amino acid 595 to about amino acid 607.

42. The recombinant factor VIII fusion protein of claim 33, wherein the surface-exposed, flexible loop structure comprising A3-1 corresponds to a region in native mature human factor VIII from about amino acid 1705 to about amino acid 1732.

43. The recombinant factor VIII fusion protein of claim 33, wherein A3-1 corresponds to a region in native mature human factor VIII from about amino acid 1711 to about amino acid 1725.

44. The recombinant factor VIII fusion protein of claim 33, wherein the surface-exposed, flexible loop structure comprising A3-2 corresponds to a region in native mature human factor VIII from about amino acid 1884 to about amino acid 1917.

45. The recombinant factor VIII fusion protein of claim 33, wherein A3-2 corresponds to a region in native mature human factor VIII from about amino acid 1899 to about amino acid 1911.

46. The recombinant factor VIII fusion protein of any one of claims 30 and 32 to 45, wherein an XTEN is inserted into at least two of the regions A1-1, A1-2, A2-1, A2-2, A3-1, or A3-2.

47. The recombinant factor VIII fusion protein of any one of claims 30 and 32 to 46, wherein an XTEN is inserted immediately downstream of an amino acid which corresponds to an amino acid in mature native human factor VIII selected from the group consisting of amino acid residue number 32, 220, 224, 399, 416, 603, 1711, 1725, 1905 and 1910.

48. The recombinant factor VIII fusion protein of any one of claims 30 and 32 to 47, wherein an additional XTEN sequence is inserted into a3 acidic spacer region.

49. The recombinant factor VIII fusion protein of claim 48, wherein the additional XTEN sequence is inserted into a3 acidic spacer immediately downstream of an amino acid which corresponds to amino acid 1656 in mature native human factor VIII.

50. The recombinant factor VIII fusion protein of claim 31, wherein the A1 domain comprises an XTEN permissive loop-1 (A1-1) region and an XTEN permissive loop-2 (A1-2) region;

wherein the A2 domain comprises an XTEN permissive loop-1 (A2-1) region and an XTEN permissive loop-2 (A2-2) region;

wherein the A3 domain comprises an XTEN permissive loop-1 (A3-1) region and an XTEN permissive loop-2 (A3-2) region; and

wherein an additional XTEN is inserted into at least one of the regions A1-1, A1-2, A2-1, A2-2, A3-1, or A3-2.

51. The recombinant factor VIII fusion protein of claim 31, wherein an additional XTEN is inserted immediately downstream of an amino acid which corresponds to an amino acid in mature native human factor VIII selected from the group consisting of amino acid residue number 32, 220, 224, 336, 339, 390, 399, 416, 603, 1656, 1711, 1725, 1905 and 1910.

52. The recombinant factor VIII fusion protein of any of claims 15 to 51, wherein the fusion protein exhibits at least about 30%, 40%, 50%, 60%, 70%, or 80%, or 90% of the procoagulant activity of the corresponding factor VIII not linked to XTEN, wherein the procoagulant activity is assayed by an in vitro coagulation assay.

53. The recombinant factor VIII fusion protein of claim 52, wherein the procoagulant activity is measured by a chromogenic assay, a one stage clotting assay or both.

54. The recombinant factor VIII fusion protein of any one of the preceding claims, wherein the fusion protein exhibits an in vitro procoagulant activity exceeding about 0.5 IU/ml, or about 1.0, or about 1.5, or about 2.0 IU/ml when expressed in cell-culture medium and assayed by an in vitro coagulation assay.

55. The recombinant factor VIII fusion protein of any one claims 30-54, wherein said fusion protein exhibits a terminal half-life at least about 3 hours, or 4 hours, or 6 hours, or 12 hours, or 13 hours, or 14 hours, or 16 hours, or 24 hours, or 48 hours, or 72 hours, or 96 hours, or 120 hours, or 144 hours, or 7 days, or 14 days, or 21 days when administered to a subject.

56. The recombinant factor VIII fusion protein of claim 55, wherein the subject is a human.

57. The recombinant factor VIII fusion protein of claim 55, wherein the subject is a factor VIII/von Willebrand factor double knock-out mouse.

58. The recombinant factor VIII fusion protein of any one of claims 30-57, wherein said fusion protein exhibits reduced binding to an anti-factor VIII antibody as compared to the corresponding factor VIII not linked to XTEN.

59. The recombinant factor VIII fusion protein of claim 58, wherein the factor VIII fusion protein has a Bethesda titer which is lower by at least about 2, 4, 6, 8, 10, 12, 15, 20, 30, 40, 50, 60, 70, 80, 100, or 200 Bethesda units compared to the factor VIII not linked to XTEN, when assayed in a Bethesda assay using an anti-factor VIII antibody selected from the group consisting of the antibodies in Table 10 and a polyclonal antibody from a hemophilia A patient with factor VIII inhibitors.

60. A recombinant factor VIII fusion protein comprising a factor VIII polypeptide and at least a first and a second extended recombinant polypeptide (XTEN), wherein the first XTEN is linked to said factor VIII polypeptide within a C2 domain of said factor VIII polypeptide, and the second XTEN is linked to said factor VIII polypeptide within an A1 or A2 domain of said factor VIII polypeptide; wherein (a) said fusion protein exhibits reduced binding to an anti-factor VIII antibody as compared to the corresponding factor VIII not linked to XTEN, the factor VIII inhibitor antibody being capable of

binding to an epitope located within the A1, A2 or C2 domain; and/or (b) wherein the fusion protein exhibits procoagulant activity.

61. The recombinant factor VIII fusion protein of claim 60, wherein the second XTEN is linked to said factor VIII polypeptide within the A2 domain of the factor VIII polypeptide.

62. The recombinant factor VIII fusion protein of claim 60, wherein the anti-factor VIII antibody binds to the A2 domain of the factor VIII polypeptide.

63. The recombinant factor VIII fusion protein of claim 60, wherein the anti-factor VIII antibody binds to the C2 domain of the factor VIII polypeptide.

64. The recombinant factor VIII fusion protein of claim 60, wherein the recombinant factor VIII fusion protein further comprises a third XTEN, wherein the third XTEN is linked to the factor VIII at an insertion site which is within or replacing the B domain or at the C-terminus.

65. The recombinant factor VIII fusion protein of claim 60 comprising a third XTEN linked within said factor VIII polypeptide at or within 1, 2, 3, 4, 5, or 6 amino acids of an insertion site selected from the group consisting of the sites in Table 7.

66. The recombinant factor VIII fusion protein of claim 60 comprising a third XTEN linked within said factor VIII polypeptide at or within 1, 2, 3, 4, 5, or 6 amino acids of an insertion site selected from the group consisting of the sites in Table 9.

67. The recombinant factor VIII fusion protein of any one of claims 60 to 66, wherein the binding of an anti-factor VIII antibody to the fusion protein is reduced by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35% or 40% compared to the corresponding factor VIII polypeptide not linked to XTEN when assayed by an ELISA assay.

68. The recombinant factor VIII fusion protein of any one of claims 60 to 67, wherein the anti-factor VIII antibody is selected from the group consisting of the antibodies in Table 10 and a polyclonal antibody from a hemophilia A subject with factor VIII inhibitors.

69. The recombinant factor VIII fusion protein of any one of claims 60 to 68, wherein the fusion protein retains procoagulant activity in the presence of an anti-factor VIII antibody of at least 10%, 20%, 30%, 40%, 50%, 80%, 100%, 200%, 300%, 400%, or 500% or more compared to the corresponding factor VIII not linked to XTEN when assayed by an in vitro coagulation assay.

70. The recombinant factor VIII fusion protein of claim 69, wherein the assay is a chromogenic assay.

71. The recombinant factor VIII fusion protein of any one of the preceding claims, each XTEN having a sequence characterized in that:

(a) the XTEN comprises at least 36 amino acid residues;

(b) the sum of glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) residues constitutes more than about 80% of the total amino acid residues of the XTEN;

(c) the XTEN sequence is substantially non-repetitive such that (i) the XTEN contains no three contiguous amino acids that are identical unless the amino acids are serine; (ii) at least about 80% of the XTEN sequence consists of non-overlapping sequence motifs, each of the sequence

motifs comprising about 9 to about 14 amino acid residues consisting of four to six amino acids selected from glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P), wherein any two contiguous amino acid residues does not occur more than twice in each of the sequence motifs; or (iii) the XTEN sequence has a subsequence score of less than 10;

(d) the XTEN has greater than 90% random coil formation as determined by GOR algorithm;

(e) the XTEN has less than 2% alpha helices and 2% beta-sheets as determined by Chou-Fasman algorithm; and

(f) the XTEN lacks a predicted T-cell epitope when analyzed by TEPITOPE algorithm, wherein the TEPITOPE threshold score for said prediction by said algorithm has a threshold of –9.

72. The recombinant factor VIII fusion protein of any one of the preceding claims, wherein each XTEN independently has at least about 80%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99%, or about 100% sequence identity compared to one or more XTEN sequences of comparable length selected from the group consisting of Table 4, Table 13, Table 14, Table 15, Table 16, and Table 17, when optimally aligned.

73. The recombinant factor VIII fusion protein of any one of the preceding claims, wherein at least one XTEN has at least 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99%, or about 100% sequence identity compared to a sequence selected from AE42_1, AE42_2, AE42_3, AG42_1, AG42_2, AG42_3, AG42_4, AE144_1A, AE144_2A, AE144_2B, AE144_3A, AE144_3B, AE144_4A, AE144_4B, AE144_5A, AE144_6B, AG144_1, AG144_2, AG144_A, AG144_B, AG144_C, AG144_F, AG144_3, AG144_4, AE288_1, AE288_2, AG288_1, and AG288_2.

74. The recombinant factor VIII fusion protein of any one of the preceding claims, comprising one, two or three amino acid substitutions in the factor VIII sequence selected from the group consisting of residues R1645, R1648, Y1680, R1689, and any combinations thereof, numbered relative to mature human factor VIII, wherein each substitution is independently to an amino acid which is alanine, glycine, or phenylalanine.

75. The recombinant factor VIII fusion protein of any one of the preceding claims, wherein the fusion protein exhibits an apparent molecular weight factor of at least about 1.3, or at least about 2, or at least about 3, or at least about 4, or at least about 5, or at least about 6, or at least about 7, or at least about 8, or at least about 9, or at least about 10.

76. The recombinant factor VIII fusion protein of any one of the preceding claims, wherein the factor VIII polypeptide is linked to a least one XTEN via one or two cleavage sequences that each is cleavable by a mammalian protease selected from the group consisting of factor XIa, factor XIIa, kallikrein, factor VIIa, factor IXa, factor Xa, factor IIa (thrombin), Elastase-2, MMP-12, MMP13, MMP-17 and MMP-20, wherein cleavage at the cleavage sequence by the mammalian protease releases the

factor VIII sequence from the XTEN, and wherein the released factor VIII sequence exhibits an increase in procoagulant activity compared to the uncleaved fusion protein.

77. The recombinant factor VIII fusion protein of claim 76, wherein the cleavage sequence(s) are cleavable by factor XIa.

78. A recombinant factor VIII fusion protein comprising at least three independent XTENs, wherein said factor VIII polypeptide comprises an A1 domain including an a1 acidic spacer region, an A2 domain including an a2 acidic spacer region, an A3 domain including an a3 acidic spacer region, a C1 domain, a C2 domain and optionally all or a portion of a B domain, and wherein each of said at least three XTENs is located at a different insertion site of the factor VIII polypeptide, wherein each of said different insertion sites is independently selected from the group consisting of:

(a) an insertion site from Table 5, Table 6, Table 7 Table 8, or Table 9;

(b) an insertion site within 6 amino acids of amino acid residue 32, 220, 224, 336, 339, 390, 399, 416, 603, 1656, 1711, 1725, 1905 or 1910 with reference to full-length human factor VIII sequence as set forth in FIG. 3;

(c) an insertion site between any two adjacent domains in the factor VIII sequence, wherein said two adjacent domains are selected from the group consisting of A1 and A2, A2 and B, B and A3, A3 and C1, and C1 and C2;

(d) an insertion site within an internal B domain deletion starting from a first position at about amino acid residue number 741 to about 750 and ending at a second position at about amino acid residue number 1635 to about 1648 with reference to full-length human factor VIII sequence as set forth in FIG. 3;

(e) the C-terminus of the factor VIII sequence;

(f) the N-terminus of the factor VIII sequence; and

(g) between two adjacent domains of said factor VIII polypeptide;

wherein the cumulative length of all XTENs is at least about 84 to about 3000 amino acid residues.

79. The recombinant factor VIII fusion protein of claim 78, wherein the fusion protein does not comprise a sequence selected from GTPGSGTASSSP (SEQ ID NO: 31), GSSTPSGATGSP (SEQ ID NO: 32), GSSPSASTGTGP(SEQ ID NO: 33), GASPGTSSTGSP(SEQ ID NO: 34), and GSEPATSGSETPGTSESATPESGPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGSEPATSGSETPGSEPATSGSETPGSEPATSGSETPGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSTEPSEGSAP (SEQ ID NO: 59).

80. The recombinant factor VIII fusion protein of claim 78 or claim 79, wherein the fusion protein does not contain an XTEN sequence consisting of

GSEPATSGSETPGTSESATPESGPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGSEPATSG
SETPGSEPATSGSETPGSEPATSGSETPGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTST
EPSEGSAP(SEQ ID NO: 59),
PGSSPSASTGTGPGSSPSASTGTGPGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGASPGTS
STGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGASPGTSSTGSPGASPGTSSTGSPGTP
GSGTASSS (SEQ ID NO: 71), or
PGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGSSTPSG
ATGSPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGA
SPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTG
SPGASPGTSSTGSPGSSTPSGATGSPGSSPSASTGTGPGASPGTSSTGSPGSSPSASTGTGPGTPGSG
TASSSPGSSTPSGATGS (SEQ ID NO: 80).

81. The recombinant factor VIII fusion protein of any one of claims 78-80, wherein the fusion protein retains at least about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90% of the procoagulant activity compared to the corresponding factor VIII not linked to XTEN, wherein the procoagulant activity is assayed by an in vitro coagulation assay.

82. The recombinant factor VIII fusion protein of any one of claims 78-80, wherein said fusion protein exhibits a prolonged terminal half-life when administered to a subject as compared to a corresponding factor VIII polypeptide lacking said XTEN.

83. The recombinant factor VIII fusion protein of claim 82, wherein said fusion protein exhibits a terminal half-life at least about 3 hours, or 4 hours, or 6 hours, or 12 hours, or 13 hours, or 14 hours, or 16 hours, or 24 hours, or 48 hours, or 72 hours, or 96 hours, or 120 hours, or 144 hours, or 7 days, or 14 days, or 21 days when administered to a subject.

84. The recombinant factor VIII fusion protein of claim 83, wherein the subject is a human.

85. The recombinant factor VIII fusion protein of claim 83, wherein the subject is a factor VIII/von Willebrand factor double knock-out mouse.

86. A recombinant factor VIII fusion protein comprising a factor VIII polypeptide and at least one XTEN, wherein said factor VIII polypeptide comprises an A1 domain, an A2 domain, an A3 domain, a C1 domain, a C2 domain and optionally all or a portion of a B domain, and wherein each of said at least one XTEN(s) is linked to said factor VIII polypeptide at an insertion site selected form the group consisting of residue numbers 18-32, or 40, or 211-224, or 336-403, or 599, or 745-1640, or 1656-1728, or 1796-1804, or 1900-1912, or 2171-2332; and wherein the fusion protein has at least about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90% of the procoagulant activity of a corresponding factor VIII not linked to XTEN.

87. A pharmaceutical composition comprising the recombinant factor VIII fusion protein of any of the preceding claims and a pharmaceutically acceptable carrier.

88. A method of treating a coagulopathy in a subject, comprising administering to said subject a composition comprising a clotting effective amount of the pharmaceutical composition of claim 87.

89. The method of claim 88, wherein after said administration, a blood concentration of procoagulant factor VIII is maintained at a greater level compared to the corresponding composition comprising factor VIII not linked to XTEN and administered to the subject at a comparable dose.

90. The method of claim 89, wherein the blood concentration is maintained at about 0.05 IU/ml, or about 0.1 IU/ml, or about 1.5 IU/ml or more for at least 48 hours after said administration.

91. A method of clotting blood in a subject, comprising contacting a clotting effective amount of the pharmaceutical composition of claim 87 with the blood.

92. A method of treating a coagulopathy in a subject with circulating inhibitors of factor VIII, comprising administering to said subject a clotting effective amount of the pharmaceutical composition of claim 87, wherein the composition exhibits greater procoagulant activity in said subject compared to a composition comprising the corresponding factor VIII not linked to XTEN and administered using a comparable amount to said subject.

93. A method of treating a bleeding episode in a subject, comprising administering to said subject a clotting effective amount of the pharmaceutical composition of claim 87, wherein the clotting effective amount of the fusion protein arrests a bleeding episode for a period that is at least two-fold, or at least three-fold longer compared to the corresponding factor VIII not linked to XTEN and administered using a comparable amount to said subject.

94. The methods of claims 88-93, wherein said coagulopathy is hemophilia A.

95. A recombinant factor VIII fusion protein for use in a pharmaceutical regimen for treatment of a hemophilia A subject, said regimen comprising a pharmaceutical composition comprising the recombinant factor VIII fusion protein of any one of claims 1-86.

96. The recombinant factor VIII fusion protein of claim 95, wherein the pharmaceutical regimen further comprises the step of determining the amount of pharmaceutical composition needed to achieve hemostasis in the hemophilia A subject.

97. The recombinant factor VIII fusion protein of claim 95, wherein the pharmaceutical regimen for treating a hemophilia A subject comprises administering the pharmaceutical composition in two or more successive doses to the subject at an effective amount, wherein the administration results in at least a 10%, or 20%, or 30%, or 40%, or 50%, or 60%, or 70%, or 80%, or 90% greater improvement of at least one, two, or three parameters associated with the hemophilia A disease compared to the factor VIII not linked to XTEN and administered using a comparable dose.

98. The recombinant factor VIII fusion protein of claim 97, wherein the parameter improved is selected from blood concentration of procoagulant factor VIII, a reduced activated partial prothrombin (aPTT) assay time, a reduced one-stage or two-stage clotting assay time, delayed onset of a bleeding episode, a reduced chromogenic assay time, a reduced bleeding assay time, resolution of a bleeding event, or a reduced Bethesda titer to native FVIII.

99. A recombinant factor VIII fusion protein used in the treatment of hemophilia A, comprising the recombinant factor VIII fusion protein of any one of claims 1 to 86.

100. A nucleic acid encoding the recombinant factor VIII fusion protein of any one of claims 1 to 86, or the complement thereof.

101. The nucleic acid of claim 100, further comprising a sequence encoding a signal peptide, wherein said sequence is ATGCAAATAGAGCTCTCCACCTGCTTCTTTCTGTGCCTTTTGCGATTCTGCTTTAGT (SEQ ID NO: 1613), or the complement thereof.

102. An expression vector comprising the nucleic acid of claim 100 or claim 101.

103. An isolated host cell comprising the expression vector of claim 102.

104. A method of producing the fusion protein of any claims 1 to 86, comprising:

(a) providing a host cell comprising an expression vector of claim 102;

(b) culturing the host cell under conditions to effect production of the fusion protein; and

(c) recovering the fusion protein.

105. A recombinant factor VIII fusion protein comprising a polypeptide having at least about 80%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99%, or at least about 100% sequence identity compared to a sequence of comparable length selected from Table 21, when optimally aligned.

106. An isolated nucleic acid comprising a polynucleotide sequence selected from (a) a sequence having at least about 80%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99%, or at least about 100% sequence identity compared to a sequence of comparable length selected from Table 21, when optimally aligned, or (b) the complement of the polynucleotide of (a).

107. The isolated nucleic acid of claim 106, further comprising the sequence ATGCAAATAGAGCTCTCCACCTGCTTCTTTCTGTGCCTTTTGCGATTCTGCTTTAGT linked to the 5' end of the nucleic acid of (a) or the complement of the sequence linked to the 3' end of (b).

CLAIMS

WHAT IS CLAIMED IS:

1. A recombinant factor VIII fusion protein comprising a factor VIII polypeptide fused to at least one extended recombinant polypeptide (XTEN), wherein the factor VIII polypeptide comprises A1 domain, A2 domain, A3 domain, a3 domain, C1 domain, C2 domain, and optionally a B domain or a portion thereof, wherein the at least one XTEN is inserted within at least one domain selected from the group consisting of the A1 domain, the A2 domain, the A3 domain, the a3 domain, the B domain or the portion thereof, the C1 domain, the C2 domain, and any combination thereof.

2. The recombinant factor VIII fusion protein of claim 1, wherein the at least one XTEN is inserted into the B domain or the portion thereof.

3. The recombinant factor VIII fusion protein of claim 1, wherein the at least one XTEN is inserted into the A1 domain, the A2 domain, the A3 domain, the a3 domain, or any combination thereof.

4. A recombinant factor VIII fusion protein of claim 1 or 3, wherein the A1 domain comprises a permissive loop-1 (A1-1) region and a permissive loop-2 (A1-2) region wherein the at least one XTEN is insereted into A1-1, A1-2, or both.

5. The recombinant factor VIII fusion protein of claim 1 or 3, wherein the A2 domain comprises a permissive loop-1 (A2-1) region and a permissive loop-2 (A2-2) region wherein the at least one XTEN is inserted into A2-1, A2-2, or both.

6. The recombinant factor VIII fusion protein of claim 1 or 3, wherein the A3 domain comprises a permissive loop-1 (A3-1) region and a permissive loop-2 (A3-2) region wherein the at least one XTEN is inserted into A3-1, A3-2, or both.

7. The recombinant factor VIII fusion protein of claim 4, wherein the permissive loop-1 (A1-1) region corresponds to a region in native mature human factor VIII from about amino acid 15 to about amino acid 45 or from about amino acid 18 to about amino acid 32 of SEQ ID NO:2, and wherein the at least one XTEN is inserted into A1-1.

8. The recombinant factor VIII fusion protein of claim 4, wherein the permissive loop-2 (A1-2) region corresponds to a region in native mature human factor VIII from about amino

acid 201 to about amino acid 232 or from about amino acid 211 to about amino acid 224 of SEQ ID NO: 2, and wherein the at least one XTEN is inserted into A1-2.

9. The recombinant factor VIII fusion protein of claim 5, wherein the permissive loop-1 (A2-1) region corresponds to a region in native mature human factor VIII from about amino acid 395 to about amino acid 421 or from about amino acid 397 to about amino acid 418 of SEQ ID NO: 2, and wherein the at least one XTEN is inserted into A2-1.

10. The recombinant factor VIII fusion protein of claim 5, wherein the permissive loop-2 (A2-2) region corresponds to a region in native mature human factor VIII from about amino acid 577 to about amino acid 635 or from about amino acid 595 to about amino acid 607 of SEQ ID NO: 2, and wherein the at least one XTEN is inserted into A2-2.

11. The recombinant factor VIII fusion protein of claim 6, wherein the permissive loop-1 (A3-1) region corresponds to a region in native mature human factor VIII from about amino acid 1705 to about amino acid 1732 or from about amino acid 1711 to about amino acid 1725 of SEQ ID NO: 2, and wherein the at least one XTEN is inserted into A3-1.

12. The recombinant factor VIII fusion protein of claim 6, wherein the permissive loop-2 (A3-2) region corresponds to a region in native mature human factor VIII from about amino acid 1884 to about amino acid 1917 or from about amino acid 1900 to about amino acid 1912 of SEQ ID NO: 2, and wherein the at least one XTEN is inserted into A3-2.

13. The recombinant factor VIII fusion protein of claim 4 or 7, wherein the at least one XTEN is inserted within A1-1 immediately downstream of an amino acid corresponding to an amino acid in mature native human factor VIII selected from the group consisting of amino acid 17 of SEQ ID NO: 2, amino acid 18 of SEQ ID NO: 2, amino acid 22 of SEQ ID NO: 2, amino acid 24 of SEQ ID NO: 2, amino acid 26 of SEQ ID NO: 2, amino acid 28 of SEQ ID NO: 2, amino acid 32 of SEQ ID NO: 2, amino acid 38 of SEQ ID NO: 2, amino acid 40 of SEQ ID NO: 2, and amino acid 41 of SEQ ID NO: 2.

14. The recombinant factor VIII fusion protein of claim 4 or 8, wherein the at least one XTEN is inserted within A1-2 immediately downstream of an amino acid corresponding to an amino acid in mature native human factor VIII selected from the group consisting of amino acid 205 of SEQ ID NO: 2, amino acid 210 of SEQ ID NO: 2, amino acid 211 of SEQ ID NO: 2, amino acid 216 of SEQ ID NO: 2, amino acid 220 of SEQ ID NO: 2, amino acid

222 of SEQ ID NO: 2, amino acid 223 of SEQ ID NO: 2, amino acid 224 of SEQ ID NO: 2, and amino acid 230 of SEQ ID NO: 2.

15. The recombinant factor VIII fusion protein of claim 5 or 9, wherein the at least one XTEN is inserted within A2-1 immediately downstream of an amino acid corresponding to an amino acid in mature native human factor VIII selected from the group consisting of amino acid 399 of SEQ ID NO: 2, amino acid 403 of SEQ ID NO: 2, amino acid 405 of SEQ ID NO: 2, amino acid 409 of SEQ ID NO: 2, and amino acid 416 of SEQ ID NO: 2.

16. The recombinant factor VIII fusion protein of claim 5 or 10, wherein the at least one XTEN is inserted within A2-2 immediately downstream of an amino acid corresponding to an amino acid in mature native human factor VIII selected from the group consisting of amino acid 598 of SEQ ID NO: 2, amino acid 599 of SEQ ID NO: 2, amino acid 603 of SEQ ID NO: 2, and amino acid 616 of SEQ ID NO: 2.

17. The recombinant factor VIII fusion protein of claim 6 or 11, wherein the at least one XTEN is inserted within A3-1 immediately downstream of an amino acid corresponding to an amino acid in mature native human factor VIII selected from the group consisting of amino acid 1711 of SEQ ID NO: 2, amino acid 1713 of SEQ ID NO: 2, amino acid 1720 of SEQ ID NO: 2, amino acid 1724 of SEQ ID NO: 2, amino acid 1725 of SEQ ID NO: 2, and amino acid 1726 of SEQ ID NO: 2.

18. The recombinant factor VIII fusion protein of any one of claim 6 or 12, wherein the at least one XTEN is inserted within A3-2 immediately downstream of an amino acid corresponding to an amino acid in mature native human factor VIII selected from the group consisting of amino acid 1896 of SEQ ID NO: 2, amino acid 1900 of SEQ ID NO: 2, amino acid 1904 of SEQ ID NO: 2, amino acid 1905 of SEQ ID NO: 2, and amino acid 1910 of SEQ ID NO: 2.

19. The recombinant factor VIII fusion protein of claim 1, wherein the at least one XTEN is inserted within a3 immediately downstream of an amino acid corresponding to an amino acid in mature native human factor VIII at amino acid 1656 of SEQ ID NO: 2.

20. The recombinant factor VIII fusion protein of claim 2, wherein the at the least one XTEN is inserted immediately downstream of amino acid 740 or 745 corresponding to an amino acid in mature native human factor VIII.

21. The recombinant factor VIII fusion protein of claim 1, wherein the at least one XTEN is inserted within the C1 or the C2 domain.

22. The recombinant factor VIII fusion protein of any one of claim 21, wherein the at least one XTEN is inserted immediately downstream of an amino acid corresponding to an amino acid in mature native human factor VIII selected from the group consisting of amino acid 2020 of SEQ ID NO: 2, amino acid 2044 of SEQ ID NO: 2, amino acid 2068 of SEQ ID NO: 2, amino acid 2073 of SEQ ID NO: 2, amino acid 2090 of SEQ ID NO: 2, amino acid 2092 of SEQ ID NO: 2, amino acid 2093 of SEQ ID NO: 2, amino acid 2111 of SEQ ID NO: 2, amino acid 2115 of SEQ ID NO: 2, amino acid 2120 of SEQ ID NO: 2, amino acid 2125 of SEQ ID NO: 2, amino acid 2171 of SEQ ID NO: 2, amino acid 2173 of SEQ ID NO: 2, amino acid 2188 of SEQ ID NO: 2, amino acid 2223 of SEQ ID NO: 2, amino acid 2224 of SEQ ID NO: 2, amino acid 2227 of SEQ ID NO: 2, amino acid 2268 of SEQ ID NO: 2, amino acid 2277 of SEQ ID NO: 2, amino acid 2278 of SEQ ID NO: 2, and amino acid 2290 of SEQ ID NO: 2.

23. The recombinant factor VIII protein of claim 1, wherein the at least one XTEN is inserted immediately downstream of an amino acid corresponding to an amino acid in mature native human factor VIII selected from the group consisting of amino acid 18 of SEQ ID NO: 2, amino acid 22 of SEQ ID NO: 2, amino acid 26 of SEQ ID NO: 2, amino acid 28 of SEQ ID NO: 2, amino acid 32 of SEQ ID NO: 2, amino acid 40 of SEQ ID NO: 2, amino acid 211 of SEQ ID NO: 2, amino acid 216 of SEQ ID NO: 2, amino acid 220 of SEQ ID NO: 2, amino acid 224 of SEQ ID NO: 2, amino acid 333 of SEQ ID NO: 2, amino acid 336 of SEQ ID NO: 2, amino acid 339 of SEQ ID NO: 2, amino acid 399 of SEQ ID NO: 2, amino acid 403 of SEQ ID NO: 2, amino acid 409 of SEQ ID NO: 2, and amino acid 416 of SEQ ID NO: 2, amino acid 599 of SEQ ID NO: 2, amino acid 603 of SEQ ID NO: 2, amino acid 1711 of SEQ ID NO: 2, amino acid 1720 of SEQ ID NO: 2, amino acid 1796 of SEQ ID NO: 2, amino acid 1802 of SEQ ID NO: 2, amino acid 1900 of SEQ ID NO: 2, amino acid 1904 of SEQ ID NO: 2, amino acid 1905 of SEQ ID NO: 2, and amino acid 1910 of SEQ ID NO: 2, amino acid 1656 of SEQ ID NO: 2 amino acid 2068 of SEQ ID NO: 2, amino acid 2171 of SEQ ID NO: 2, amino acid 2227 of SEQ ID NO: 2, and amino acid 2277 of SEQ ID NO: 2.

24. The recombinant factor VIII fusion protein of any one of claims 1-23, comprising at least two XTENs.

25. The recombinant factor VIII fusion protein of claim 24, wherein the at least two XTENs are inserted or fused immediately downstream of one or two amino acids corresponding to an amino acid in mature native human factor VIII selected from the group consisting of amino acid 18 of SEQ ID NO: 2, amino acid 26 of SEQ ID NO: 2, amino acid 40 of SEQ ID NO: 2, amino acid 399 of SEQ ID NO: 2, amino acid 403 of SEQ ID NO: 2, amino acid 599 of SEQ ID NO: 2, amino acid 745 of SEQ ID NO: 2, amino acid 1656 of SEQ ID NO: 2, amino acid 1711 of SEQ ID NO: 2, amino acid 1720 of SEQ ID NO: 2, amino acid 1725 of SEQ ID NO: 2, amino acid 1900 of SEQ ID NO: 2, amino acid 1905 of SEQ ID NO: 2, and amino acid 2332 of SEQ ID NO: 2.

26. The recombinant factor VIII fusion protein of claim 24 or 25, wherein the at least two XTENs are inserted or fused immediately downstream of the amino acids corresponding to the amino acids in mature native human factor VIII selected from the group consisting of

i. amino acids 745 and 2332 of SEQ ID NO: 2;
ii. amino acids 1656 and 2332 of SEQ ID NO: 2;
iii. amino acids 26 and 403 of SEQ ID NO: 2;
iv. amino acids 40 and 403 of SEQ ID NO: 2;
v. amino acids 18 and 403 of SEQ ID NO: 2;
vi. amino acids 26 and 599 of SEQ ID NO: 2;
vii. amino acids 40 and 599 of SEQ ID NO: 2;
viii. amino acids 18 and 599 of SEQ ID NO: 2;
ix. amino acids 18 and 1656 of SEQ ID NO: 2;
x. amino acids 26 and 1656 of SEQ ID NO: 2;
xi. amino acids 40 and 1656 of SEQ ID NO: 2;
xii. amino acids 403 and 1656 of SEQ ID NO: 2;
xiii. amino acids 1656 and 1720 of SEQ ID NO: 2;
xiv. amino acids 40 and 399 of SEQ ID NO: 2;
xv. amino acids 26 and 1900 of SEQ ID NO: 2;
xvi. amino acids 18 and 399 of SEQ ID NO: 2;
xvii. amino acids 40 and 399 of SEQ ID NO: 2; and
xviii. amino acids 1656 and 1900 of SEQ ID NO: 2.

27. The recombinant factor VIII fusion protein of any one of claims 1 to 26, comprising at least three XTENs, at least four XTENs, at least five XTENs, or at least six XTENs.

28. The recombinant factor VIII fusion protein of claim 27, wherein

i. the at least three XTENs are inserted or fused immediately downstream of one or more amino acids corresponding to an amino acid in mature native human factor VIII selected from the group consisting of amino acid 18 of SEQ ID NO: 2, amino acid 26 of SEQ ID NO: 2, amino acid 40 of SEQ ID NO: 2, amino acid 399 of SEQ ID NO: 2, amino acid 403 of SEQ ID NO: 2, amino acid 599 of SEQ ID NO: 2, amino acid 745 of SEQ ID NO: 2, amino acid 1656 of SEQ ID NO: 2, amino acid 1711 of SEQ ID NO: 2, amino acid 1720 of SEQ ID NO: 2, amino acid 1725 of SEQ ID NO: 2, amino acid 1900 of SEQ ID NO: 2, amino acid 1905 of SEQ ID NO: 2, amino acid 1910 of SEQ ID NO: 2, and amino acid 2332 of SEQ ID NO: 2;

ii. the at least four XTENs are inserted or fused immediately downstream of one or more amino acids corresponding to an amino acid in mature native human factor VIII selected from the group consisting of amino acid 18 of SEQ ID NO: 2, amino acid 26 of SEQ ID NO: 2, amino acid 40 of SEQ ID NO: 2, amino acid 403 of SEQ ID NO: 2, amino acid 409 of SEQ ID NO: 2, amino acid 745 of SEQ ID NO: 2, amino acid 1656 of SEQ ID NO: 2, amino acid 1720 of SEQ ID NO: 2, amino acid 1900 of SEQ ID NO: 2, amino acid 1905 of SEQ ID NO: 2, amino acid 1910 of SEQ ID NO: 2, and amino acid 2332 of SEQ ID NO: 2; or

iii. the at least five XTENs are inserted or fused immediately downstream of one or more amino acids corresponding to an amino acid in mature native human factor VIII selected from the group consisting of amino acid 18 of SEQ ID NO: 2, amino acid 403 of SEQ ID NO: 2, amino acid 745 of SEQ ID NO: 2, amino acid 1656 of SEQ ID NO: 2, amino acid 1720 of SEQ ID NO: 2, amino acid 1900 of SEQ ID NO: 2, and amino acid 2332 of SEQ ID NO: 2.

29. The recombinant factor VIII fusion protein of any one of claims 1-28, wherein the at least one XTEN comprises at least 36 amino acids, at least 42 amino acids, at least 72 amino acids, at least 96 amino acids, at least 144 amino acids, or at least 288 amino acids.

30. The recombinant factor VIII fusion protein of claim 29, wherein the at least one XTEN comprises at least 72 amino acids, at least 144 amino acids, or at least 288 amino acids.

31. The recombinant factor VIII fusion protein of any one of claims 1-30, wherein the at least one XTEN comprises one or more XTEN sequence motifs, which are selected from the group consisting of SEQ ID NOs: 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, and 48.

32. The recombinant factor VIII fusion protein of claim 31, wherein the one or more XTEN sequence motifs are selected from the group consisting of SEQ ID NOs: 23, 24, 25, and 26.

33. The recombinant factor VIII fusion protein of any one of claims 1-32, wherein the at least one XTEN comprises an amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 49, 50, 51, 52, 57, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 78, and 79.

34. The recombinant factor VIII fusion protein of any one of claims 1-32, wherein the at least one XTEN comprises an amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, and 351.

35. The recombinant factor VIII fusion protein of any one of claims 1-34, wherein the factor VIII polypeptide comprises a native factor VIII polypeptide.

36. The recombinant factor VIII fusion protein of any one of claims 1-35, wherein the factor VIII polypeptide comprises a partially deleted B domain or a fully deleted B domain.

37. The recombinant factor VIII fusion protein of any one of claims 1-36, wherein the factor VIII polypeptide comprises a single chain factor VIII polypeptide.

38. The recombinant factor VIII fusion protein of any one of claims 1-37, wherein at least one heterologous polypeptide is inserted within the A1 domain, the A2 domain, the A3 domain, the B domain, the a3 domain, the C1 domain, the C2 domain, or fused to the C-terminus of the factor VIII polypeptide.

39. The recombinant factor VIII fusion protein of claim 38, wherein the at least one heterologous polypeptide is an Fc fragment of immunoglobulin or an FcRn binding domain.

40. The recombinant factor VIII fusion protein of claim 38, wherein the at least one heterologous polypeptide is selected from the group consisting of albumin or a fragment thereof, a β subunit of C-terminal peptide of human chorionic gonadotropin, a HAP sequence, a transferrin, a PAS polypeptide, a polyglycine linker, a polyserine linker, and a combination thereof.

41. An isolated nucleic acid encoding the recombinant factor VIII fusion protein of any one of claims 1-40.

42. A vector comprising the isolated nucleic acid of claim 41.

43. The vector of claim 42, wherein the vector is a plasmid, a cosmid, a viral particle, or phage.

44. A host cell comprising the vector of claim 42 or 43.

45. A pharmaceutical composition comprising the recombinant factor VIII fusion protein of any one of claims 1-40 and a pharmaceutically acceptable carrier.

46. A pharmaceutical composition comprising the isolated nucleic acid of claim 41, the vector of claim 42 or 43, or the host cell of claim 44, and a pharmaceutically acceptable carrier.

47. A method of making a recombinant factor VIII fusion protein comprising culturing the host cell of claim 44 in media under suitable conditions.