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DESCRIPTION

BACKGROUND OF THE INVENTION

[0001] Haemophilia A is a bleeding disorder caused by defects in the gene encoding coagulation factor VIII (FVIII) and affects 1-2 in 10,000 male births. Graw et al., Nat. Rev. Genet. 6(6): 488-501 (2005). Patients affected with hemophilia A can be treated with infusion of purified or recombinantly produced FVIII. All commercially available FVIII products, however, are known to have a half-life of about 8-12 hours, requiring frequent intravenous administration to the patients. *See* Weiner M.A. and Cairo, M.S., Pediatric Hematology Secrets, Lee, M.T., 12. Disorders of Coagulation, Elsevier Health Sciences, 2001; Lillicrap, D. Thromb. Res. 122 Suppl 4:S2-8 (2008). In addition, a number of approaches have been tried in order to extend the FVIII half-life. For example, the approaches in development to extend the half-life of clotting factors include pegylation, glycopegylation, and conjugation with albumin. See Dumont et al., Blood. 119(13): 3024-3030 (Published online Jan. 13, 2012). Regardless of the protein engineering used, however, the long acting FVIII products currently under development are reported to have limited half-lives - only to about 1.5 to 2 hours in preclinical animal models. *See id.* Consistent results have been demonstrated in humans, for example, rFVIIIFc was reported to improve half-life up to ~ 1.7 fold compared with ADVATE® in hemophilia A patients. *See Id.* Therefore, the half-life increases, despite minor improvements, may indicate the presence of other T1/2 limiting factors. *See* Liu, T. *et al.,* 2007 ISTH meeting, abstract #P-M-035; Henrik, A. *et al.,* 2011 ISTH meeting, abstract #P=MO-181; Liu, T. *et al.,* 2011 ISTH meeting abstract #P-WE-131.

[0002] Plasma von Willebrand Factor (VWF) has a half-life of approximately 16 hours (ranging from 13 to 18 hours). Goudemand J, et al. J Thromb Haemost 2005;3:2219-27. The VWF half-life may be affected by a number of factors: glycosylation pattern, ADAMTS-13 (a disintegrin and metalloprotease with thrombospondin motif-13), and various mutations in VWF.

[0003] In plasma, 95-98% of FVIII circulates in a tight non-covalent complex with full-length VWF. The formation of this complex is important for the maintenance of appropriate plasma levels of FVIII in vivo. Lenting et al., Blood. 92(11): 3983-96 (1998); Lenting et al., J. Thromb. Haemost. 5(7): 1353-60 (2007). The full-length wild-type FVIII is mostly present as a heterodimer having a heavy chain (MW 200kD) and a light chain (MW 73kD). When FVIII is activated due to proteolysis at positions 372 and 740 in the heavy chain and at position 1689 in the light chain, the VWF bound to FVIII is removed from the activated FVIII. The activated FVIII, together with activated factor IX, calcium, and phospholipid ("tenase complex"), induces the activation of factor X, generating large amounts of thrombin. Thrombin, in turn, then cleaves fibrinogen to form soluble fibrin monomers, which then spontaneously polymerize to form the soluble fibrin polymer. Thrombin also activates factor XIII, which, together with calcium, serves to crosslink and stabilize the soluble fibrin polymer, forming crosslinked (insoluble) fibrin. The activated FVIII is cleared fast from the circulation by proteolysis.

[0004] Due to the frequent dosing and inconvenience caused by the dosing schedule, there is still a need to develop FVIII products requiring less frequent administration, *i.e.,* a FVIII product that has a half-life longer than the 1.5 to 2 fold half-life limitation.

BRIEF SUMMARY OF THE INVENTION

[0005] The present invention provides a chimeric protein comprising

1. (i) a first polypeptide which comprises
    1. (a) a Factor VIII ("FVIII") protein,
    2. (b) an XTEN of the first polypeptide, and

3. (c) a first Fc region,

wherein the FVIII protein has a FVIII activity; and

2. (ii) a second polypeptide, which comprises
    1. (a) a von Willebrand Factor ("VWF") fragment comprising an amino acid sequence at least 99% identical to the amino acid sequence set forth in amino acids 764 to 1240 of SEQ ID NO: 21,
    2. (b) an XTEN of the second polypeptide,
    3. (c) a 10 to 50 amino acid cleavable linker comprising an a2 region of FVIII which comprises the amino acid sequence of Glu720 to Arg740 corresponding to SEQ ID NO: 65, wherein the a2 region is capable of being cleaved by thrombin, and
    4. (d) a second Fc region,

wherein the VWF fragment binds to the FVIII protein;

wherein the XTEN of the second polypeptide comprises the amino acid sequence as set forth in SEQ ID NO: 58;

wherein the XTEN of the first polypeptide comprises the amino acid sequence as set forth in SEQ ID NO: 8;

wherein the first Fc region is associated with the second Fc region by a disulfide bond; and wherein the half-life of the chimeric protein is extended compared to a FVIII protein without the VWF fragment.

[0006] The present invention also provides a chimeric protein comprising

1. (i) a first polypeptide, which comprises
    1. (a) a Factor VIII ("FVIII") protein,
    2. (b) an XTEN of the first polypeptide, and
    3. (c) a first Fc region,

wherein the FVIII protein has a FVIII activity; and

2. (ii) a second polypeptide, which comprises
    1. (a) a von Willebrand Factor ("VWF") fragment comprising an amino acid sequence at least 99% identical to the amino acid sequence set forth in amino acids 764 to 1240 of SEQ ID NO: 21,
    2. (b) an XTEN of the second polypeptide,
    3. (c) a 10 to 50 amino acid cleavable linker comprising an a2 region of FVIII which comprises the amino acid sequence of Glu720 to Arg740 corresponding to SEQ ID NO: 65, wherein the a2 region is capable of being cleaved by thrombin, and
    4. (d) a second Fc region,

wherein the VWF fragment binds to the FVIII protein,

wherein the VWF fragment contains an amino acid other than cysteine substituted for a residue corresponding to residues 1099 and 1142 of SEQ ID NO: 21;

wherein the XTEN of the second polypeptide comprises the amino acid sequence as set forth in SEQ ID NO: 58;

wherein the XTEN of the first polypeptide comprises the amino acid sequence as set forth in SEQ ID NO: 8;

wherein the first Fc region is associated with the second Fc region by a disulfide bond; and wherein the half-life of the chimeric protein is extended compared to a FVIII protein without the VWF fragment.

[0007] In certain embodiments the first polypeptide further comprises a second XTEN sequence which links the FVIII protein with the first Fc region. Also disclosed is the chimeric protein as described herein, wherein the first polypeptide comprises a third XTEN sequence which is inserted at one or more insertion sites within the FVIII protein. In some embodiments the first polypeptide further comprises a second XTEN sequence which is inserted at one or more insertion sites within the FVIII protein. In certain embodiments, the first polypeptide

comprises a third XTEN sequence which links the FVIII protein with the first Fc region.

[0008] Also disclosed is the chimeric protein as described herein, wherein the second XTEN sequence, the third XTEN sequence, or the second and third XTEN sequences are each independently selected from AE42, AE72, AE864, AE576, AE288, AE144, AG864, AG576, AG288, and AG144. In some embodiments the second XTEN sequence, the third XTEN sequence, or the second and third XTEN sequences are each independently selected from SEQ ID NO: 8; SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 17; SEQ ID NO: 54; SEQ ID NO: 19; SEQ ID NO: 16; SEQ ID NO: 18; SEQ ID NO: 15; SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 14; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; or SEQ ID NO: 63. In certain embodiments the second XTEN sequence, the third XTEN sequence, or both the second and third XTEN sequences are each independently AE288 or AG288.

[0009] The first Fc region and the second Fc region may extend the half-life of the chimeric protein. In some embodiments the first polypeptide and the second polypeptide is fused by a linker. In certain embodiments the first polypeptide and the second polypeptide is fused by a processable linker. In some embodiments the first Fc region is associated with the second Fc region. In certain embodiments the first Fc region is associated with the second Fc region by a covalent bond. In some embodiments the covalent bond is a disulfide bond.

[0010] In certain embodiments the linker comprises a fragment of the a2 region of FVIII. The fragment of the a2 region can in some cases comprise the sequence DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88). In one particular embodiment, the linker comprises the amino acid sequence of IEPRSFS (SEQ ID NO: 194). In another embodiment, the linker comprises the amino acid sequence of IEPRSFS (SEQ ID NO: 194), wherein the linker is not the full-length a2 region of FVIII.

[0011] Also disclosed is the chimeric protein as described herein, wherein the a2 region of FVIII comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to either ISDKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 106) or DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88).

[0012] Also disclosed is the chimeric protein as described herein, wherein the half-life of the chimeric protein is at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 25 hours, at least about 26 hours, at least about 27 hours, at least about 28 hours, at least about 29 hours, at least about 30 hours, at least about 31 hours, at least about 32 hours, at least about 33 hours, at least about 34 hours, at least about 35 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours. In some embodiments the half-life of the chimeric protein is about 40 hours in HemA mice. In certain embodiments the VWF fragment does not bind substantially to a VWF clearance receptor. In some embodiments the VWF fragment is capable of protecting the FVIII protein from one or more protease cleavages, protecting the FVIII protein from activation, stabilizing the heavy chain and/or the light chain of the FVIII protein, or preventing clearance of the FVIII protein by one or more scavenger receptors.

[0013] Some embodiments include the chimeric protein as described herein, wherein the VWF fragment inhibits or prevents endogenous VWF from binding to the FVIII protein by shielding or blocking a VWF binding site on the FVIII protein. In certain embodiments the VWF binding site is located in the A3 domain or the C2 domain of the FVIII protein or both the A3 domain and the C2 domain. In some embodiments the VWF binding site comprises the amino acid sequence corresponding to amino acids 1669 to 1689 and 2303 to 2332 of SEQ ID NO: 65. In some embodiments the first Fc region and the second Fc region are identical or different.

[0014] Also disclosed is the chimeric protein as described herein, wherein the FVIII protein comprises one or more domains of FVIII selected from an A1 domain, a1 acidic region, an A2 domain, a2 acidic region, a B domain, an A3 domain, a3 acidic region, a C1 domain, a C2 domain, one or more fragments thereof, and any

combinations thereof.

[0015] Also disclosed is the chimeric protein as described herein, wherein the one or more insertion sites in the FVIII protein is located within one or more domains of the FVIII protein selected from the A1 domain, the a1 acidic region, the A2 domain, the a2 acidic region, the A3 domain, the B domain, the C1 domain, the C2 domain, and any combinations thereof or between one or more domains of the FVIII protein selected from the group consisting of the A1 domain and a1 acidic region, the a1 acidic region and A2 domain, the A2 domain and a2 acidic region, the a2 acidic region and B domain, the B domain and A3 domain, the A3 domain and C1 domain, the C1 domain and C2 domain, and any combinations thereof or between two domains of the FVIII protein selected from the A1 domain and a1 acidic region, the a1 acidic region and A2 domain, the A2 domain and a2 acidic region, the a2 acidic region and B domain, the B domain and A3 domain, the A3 domain and C1 domain, the C1 domain and C2 domain, and any combinations thereof. In some embodiments the one or more insertion sites in the FVIII protein are one or more amino acids selected from the group consisting of the amino acid residues in Table 7, Table 8, Table 9 and Table 10. In certain embodiments the insertion sites in the FVIII protein are located immediately downstream of amino acid 745 corresponding to the mature FVIII protein (SEQ ID NO: 65). In some embodiments the insertion sites in the FVIII protein are located immediately downstream of residue 1656 and residue 1900 corresponding to the mature FVIII protein (SEQ ID NO: 65). In some embodiments the insertion sites in the FVIII protein are immediately downstream of residues 26, 1656, and 1900 corresponding to the mature FVIII protein (SEQ ID NO: 65). In certain embodiments the insertion sites in the FVIII protein are immediately downstream of residues 403 and 745 corresponding to the mature FVIII protein (SEQ ID NO: 65). In some embodiments the insertion sites in the FVIII protein are immediately downstream of residues 745 and 1900 corresponding to the mature FVIII protein (SEQ ID NO: 65). In certain embodiments the insertion sites in the FVIII protein are immediately downstream of residues 18 and 745 corresponding to the mature FVIII protein (SEQ ID NO: 65). In some embodiments the FVIII protein is a dual chain FVIII isoform. In some embodiments the FVIII protein is a single chain FVIII isoform. In certain embodiments the FVIII protein comprises B domain or a portion thereof. In some embodiments the FVIII protein is SQ B domain deleted FVIII.

[0016] Some embodiments include the chimeric protein as described herein, wherein the single chain FVIII isoform contains at least one amino acid substitution at a residue corresponding to residue 1648, residue 1645, or both residues corresponding to the full-length mature Factor VIII polypeptide (SEQ ID NO: 65) or residue 754, residue 751, or both residues of SQ BDD Factor VIII (SEQ ID NO: 67). In certain embodiments the amino acid substitution is an amino acid other than arginine. In some embodiments the dual chain FVIII isoform comprises a first chain comprising a heavy chain of FVIII and a second chain comprising a light chain of FVIII, wherein the heavy chain and the light chain are associated with each other by a metal bond. Disclosed herein is a D' domain which may comprise an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 866 of SEQ ID NO: 21. Also disclosed herein is a D3 domain which may comprise an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acids 867 to 1240 of SEQ ID NO: 21. The VWF protein may be a monomer.

[0017] Also disclosed is the chimeric protein as described herein, which comprises at least two VWF proteins, at least three VWF proteins, at least four VWF proteins, at least five VWF proteins, or at least six VWF proteins. Disclosed herein are VWF proteins which may comprise an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 1240 of SEQ ID NO: 21. In some embodiments the VWF protein consists essentially of or consists of amino acids 764 to 1240 of SEQ ID NO: 21. A VWF protein may contain at least one amino acid substitution at a residue corresponding to residue 1099, residue 1142, or both residues 1099 and 1142 of SEQ ID NO: 21. A VWF protein may contain an amino acid other than cysteine substituted for a residue corresponding to residue 1099, residue 1142, or both residues 1099 and 1142 of SEQ ID NO: 21. In a particular embodiment of the invention, the VWF fragment contains an alanine residue substituted for the residues corresponding to residues 1099 and 1142 of SEQ ID NO: 21. A VWF protein may further comprise the D1 domain, the D2 domain, or the D1 and D2 domains of VWF.

[0018] In the chimeric protein as described herein, the VWF protein may further comprise a VWF domain

selected from the A1 domain, the A2 domain, the A3 domain, the D4 domain, the B1 domain, the B2 domain, the B3 domain, the C1 domain, the C2 domain, the CK domain, one or more fragments thereof, and any combinations thereof.

[0019] Also disclosed is the chimeric protein as described herein, wherein the VWF protein consists essentially of or consists of: (1) the D' and D3 domains of VWF or fragments thereof; (2) the D1, D', and D3 domains of VWF or fragments thereof; (3) the D2, D', and D3 domains of VWF or fragments thereof; (4) the D1, D2, D', and D3 domains of VWF or fragments thereof; or (5) the D1, D2, D', D3, and A1 domains of VWF or fragments thereof.

[0020] Also disclosed is the chimeric protein as described herein, wherein the VWF protein further comprises a signal peptide of VWF or FVIII which is operably linked to the VWF protein.

[0021] In some embodiments, the chimeric protein as described herein is polysialylated, pegylated, or hesylated.

[0022] Also disclosed is the chimeric protein as described herein, wherein the first polypeptide comprises at least about 80%, 90%, 95%, 99%, or 100% identical to FVIII161 (SEQ ID NO: 69), FVIII169 (SEQ ID NO: 70), FVIII173 (SEQ ID NO: 72), FVIII195 (SEQ ID NO: 73), FVIII196 (SEQ ID NO: 74), FVIII199 (SEQ ID NO: 75), FVIII201 (SEQ ID NO: 76), FVIII203 (SEQ ID NO: 77), FVIII204 (SEQ ID NO: 78), FVIII205 (SEQ ID NO: 79), FVIII266 (SEQ ID NO: 80), FVIII267 (SEQ ID NO: 81), FVIII268 (SEQ ID NO: 82), FVIII269 (SEQ ID NO: 83), FVIII271 (SEQ ID NO: 84), FVIII272 (SEQ ID NO: 85), or FVIII282 (SEQ ID NO: 159), and the second polypeptide comprises at least about 80%, 90%, 95%, 99%, or 100% identical to VWF059 (SEQ ID NO: 197).. In other embodiments, the first polypeptide comprises FVIII169 (SEQ ID NO: 70) and the second polypeptide comprises VWF059 (SEQ ID NO: 197). In some embodiments, the chimeric protein is efficacious in preventing and/or stopping bleeding from a subject in need thereof.

[0023] Also disclosed is a polynucleotide or a set of polynucleotides encoding the chimeric protein as described herein. In some embodiments, the polynucleotide as described herein, further comprises a polynucleotide chain, which encodes PC5 or PC7.

[0024] Some embodiments include a vector comprising the polynucleotide as described herein and one or more promoter operably linked to the polynucleotide or the set of polynucleotides.

[0025] In some embodiments the vector as described herein, further comprises an additional vector, which comprises a polynucleotide chain encoding PC5 or PC7.

[0026] Also disclosed is a host cell comprising the polynucleotide or the vector as described herein. In some embodiments the host cell is a mammalian cell. In certain embodiments the mammalian cell is selected from HEK293 cell, CHO cell, and BHK cell.

[0027] Also disclosed is a pharmaceutical composition comprising the chimeric protein and a pharmaceutically acceptable carrier. In some embodiments the chimeric protein has extended half-life compared to wild type FVIII protein. In certain embodiments, the half-life of the chimeric protein is extended at least about 1.5 times, at least about 2 times, at least about 2.5 times, at least about 3 times, at least about 4 times, at least about 5 times, at least about 6 times, at least about 7 times, at least about 8 times, at least about 9 times, at least about 10 times, at least about 11 times, or at least about 12 times longer than wild type FVIII.

[0028] Some embodiments include the composition as described herein, wherein the half-life of the chimeric protein is at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 25 hours, at least about 26 hours, at least about 27 hours, at least about 28 hours, at least about 29 hours, at least about 30 hours, at least about 31 hours, at least about 32 hours, at least about 33 hours, at least about 34

hours, at least about 35 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours. In certain embodiments the half-life of the chimeric protein is about 40 hours in HemA mice. In some embodiments the composition as described herein is administered by a route selected from the group consisting of topical administration, intraocular administration, parenteral administration, intrathecal administration, subdural administration and oral administration. In certain embodiments the parenteral administration is intravenous or subcutaneous administration. In some embodiments the composition as described herein is used to treat a bleeding disease or condition in a subject in need thereof. In certain embodiments the bleeding disease or condition is selected from the group consisting of a bleeding coagulation disorder, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, bleeding in the iliopsoas sheath and any combinations thereof. In some embodiments the subject is scheduled to undergo a surgery. In certain embodiments the treatment is prophylactic or on-demand.

[0029] Also disclosed is a method of extending or increasing half-life of the chimeric protein, wherein the method comprises adding an effective amount of the chimeric proteinor the composition as described herein to a subject in need thereof, wherein the VWF protein, the XTEN sequence, the first Ig constant region or a portion thereof, and the second Ig constant region or a portion thereof increase the half-life of the chimeric protein.

[0030] Some embodiments include the chimeric protein or the composition as described herein for use in a method of treating a bleeding disease or disorder in a subject in need thereof comprising administering an effective amount of the chimeric protein or the composition as described herein, wherein the bleeding disease or disorder is selected from the group consisting of a bleeding coagulation disorder, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, and bleeding in the iliopsoas sheath. In some embodiments the subject is an animal. In certain embodiments the animal is a human. In some embodiments the subject is suffering from hemophilia A. In certain embodiments the treatment is prophylactic or on-demand. In some embodiments the effective amount is 0.1 μg/kg to 500 mg/kg.

[0031] Also disclosed is a method as described herein, wherein the chimeric protein or the composition as described herein is administered by a route selected from the group consisting of topical administration, intraocular administration, parenteral administration, intrathecal administration, subdural administration and oral administration. In certain embodiments the parenteral administration is selected from the group consisting of intravenous administration, subcutaneous administration, intramuscular administration, and intradermal administration.

[0032] Also disclosed is a method of making a chimeric protein, comprising transfecting one or more host cell with the polynucleotide or the vector as described herein and expressing the chimeric protein in the host cell. The method as described herein may further comprise isolating the chimeric protein. The chimeric protein is efficacious in stopping and/or preventing bleeding in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0033]

Figure 1 shows a schematic diagram of a chimeric protein comprising a first polypeptide which comprises a FVIII protein (A1-A2-partial or full B-A3-C1-C2) fused to an Fc region, wherein an XTEN is inserted at an insertion site within the FVIII protein and a second polypeptide which comprises a VWF protein comprising D'D3 domains, an XTEN having less than 288 amino acids, a thrombin cleavable linker, and a second Fc region. XTEN insertions in

the FVIII protein and/or fusions to the VWF protein extend a half-life of the chimeric protein by increasing the hydrodynamic radius and by blocking receptor-mediated clearance. The D'D3 domains of VWF block FVIII interaction with endogenous VWF, stabilize the FVIII protein, and extend a half-life of the chimeric protein. The Fc domains can covalently link the D'D3 domains with the FVIII protein and extend a half-life of the chimeric protein through FcRn-mediated recycling pathway. The thrombin-cleavable linker enables a release of the D'D3 domains upon FVIII activation and ensures the correct alignment between FVIII and the D'D3 domains of VWF.

Figure 2 shows three plasmid expression system for FVIII-XTEN-Fc:D'D3-XTEN-Fc heterodimers: a first plasmid comprising a nucleotide sequence encoding single chain FVIII-XTEN-Fc in which an XTEN is inserted in the B domain; a second plasmid comprising a nucleotide sequence encoding D1D2D'D3-XTEN-Fc, in which the XTEN sequence comprises less than 288 amino acids; and a third plasmid comprising a nucleotide sequence encoding PACE, a propeptide processing enzyme. When the three polypeptides are expressed from the three plasmids, the D1D2 propeptide domains of VWF can be processed from the D'D3 domains by intracellular processing. The resulting complex contains three products, the first molecule being FVIII-XTEN/D'D3 heterodimers, the second molecule being a by-product, homodimer of D'D3-XTEN-Fc, and the third molecule being another by-product, i.e., FVIII(XTEN)-Fc.

Figure 3 shows additive effects of XTEN insertions on the half-life extension of the heterodimers. FVIII169 comprises a B domain deleted FVIII protein fused to an Fc region, wherein an XTEN sequence (e.g., AE288) is inserted at amino acid 745 corresponding to mature full length FVIII. FVIII205 comprises a B domain deleted FVIII protein fused to an Fc region, wherein an XTEN sequence (e.g., a 144XTEN) is inserted at amino acid 18 corresponding to mature full length FVIII and another XTEN sequence (e.g., AE288) is inserted at amino acid 745 corresponding to mature full length FVIII. VWF031 comprises a D' domain and a D3 domain of VWF fused to an Fc region by a thrombin cleavable linker (no XTEN). VWF034 comprises a D' domain and a D3 domain of VWF fused to AE288 and an Fc region. The half-life of FVIII169/VWF031 (inverted triangle) is 16.7 hours in HemA mice; the half-life of FVIII205/VWF031 (circle) is 29.4 hours in HemA mice; and the half-life of FVIII169/VWF034 (square) is 31.1 hours in HemA mice.

Figure 4 shows that a 144 amino acid XTEN confers better half-life extension than a 288 amino acid XTEN when inserted between the D'D3 domains of VWF and Fc domains. For example, while the half-life of VWF169/VWF034 (square) is 31.1. hours in HemA mice, the half-life of FVIII169/VWF057 (circle) is 42 hours in HemA mice. VWF057 comprises D'D3 domains of VWF fused to a 144 amino acid XTEN and an Fc region.

Figure 5 shows that Fc domains are needed for half-life extension of the chimeric protein heterodimers. When the half-life of FVIII205/VWF031 (circle) was compared in HemA mice with that of FVIII263/VWF050 (square), which contains mutations at the FcRn binding sites (IHH triple mutation Fc) and thus cannot be recycled through FcRn pathway, the half-life of FVIII263/VWF050 (23 hours) is shorter than that of VWF205/VWF031 (29.4 hours). This indicates that the Fc regions are necessary for half-life extension.

Figure 6A shows similar acute efficacy of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers compared to B domain deleted FVIII (SQ BDD FVIII) in HemA mice tail clip model. Mice were dosed at 75 IU/kg, and the activity was measured by aPTT assay. SQ BDD FVIII is shown as circle while FVIII169/VWF034 is shown as square, FVIII169/VWF057 is shown as diamond, and vehicle is shown as inverted triangle. The construct details of FVIII169, VWF034, and VWF057 are shown elsewhere herein. Figure 6B shows a comparison of the acute efficacy of FVIII169/VWF034 with B domain deleted FVIII (SQ BDD FVIII) in HemA mice at 37.5 IU/kg dose, and the activity was measured by aPTT assay. The median blood loss (uL) of mice in each treatment groups are indicated by the horizontal lines, blood loss (uL) in C57/BL6 mice is shown as hollow triangle; the blood loss (uL) after dosing of 37.5 IU/kg of rBDD-FVIII is shown as hollow circle; the blood loss (uL) after dosing of 37.5 IU/kg FVIII169/VWF034 is shown as hollow square and the blood loss (uL) after dosing of vehicle is shown as inverted triangle.

Figures 7A-B show that rFVIII169/VWF057 heterodimer provides longer protection to HemA mice in Tail Vein Transection Bleeding Model. Figure 7A shows the rebleeding data in mice that received rFVIII169/VWF057 at 72 hours before tail injury (square), SQ BDD-FVIII at 48 hours before tail injury (diamond), SQ BDD FVIII at 24

hours before tail injury (inverted triangle), and vehicle (circle). The activity was measured by aPTT assay. X-axis shows time in hours, and the Y axis shows percent of Non-Bleeders. Figure 7B shows the corresponding survival data in the four categories of the mice shown in Figure 7A. The mice received 12 IU/kg of FVIII169/VWF057 72 hours prior to tail injury showed similar protection on re-bleeding and survival compared to the mice received SQ BDD FVIII treatment 24 hour before the tail injury.

Figure 8A shows the comparable rebleeding data in mice that received rFVIII-XTEN-Fc/D'D3-XTEN-Fc Heterodimers at 96 hours versus rBDD-FVIII at 24 hours before the injury. Filled squares show the rebleeding data in mice received FVIII169/VWF034 at 24 hours before the injury; hollow squares show the rebleeding data in mice received FVIII169/VWF034 at 96 hours before the injury; filled diamond show the rebleeding data in mice received FVIII169/VWF057 at 24 hours before the injury; hollow diamond show the rebleeding data in mice received FVIII169/VWF057 at 96 hours before the injury; filled circles show the rebleeding data in mice received rBDD-FVIII at 24 hours before the injury; hollow circles show the rebleeding data in mice received rBDD-FVIII at 48 hours before the injury; and filled triangle show the rebleeding data in mice received vehicle. X axis shows time in hours, and y axis shows percent of Non-Bleeders

Figure 8B shows the survival curve in mice that received rFVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers at 96 hours versus rBDD-FVIII at 24 hours before the injury. X axis shows time in hours, and y axis shows percent of survival. The symbols are the same as Figure 8A.

Figure 9 shows a diagram of representative FVIII-VWF heterodimers and FVIII169, FVIII286, VWF057, VWF059, and VWF062 constructs. For example, FVIII169 construct comprises a B domain deleted FVIII protein with R1648A substitution fused to an Fc region, wherein an XTEN sequence *(e.g.,* AE288) is inserted at amino acid 745 corresponding to mature full length FVIII (A1-a1-A2-a2-288XTEN-a3-A3-C1-C2-Fc). FVIII286 construct comprises a B domain deleted FVIII protein with R1648 substitution fused to an Fc region, wherein an XTEN sequence *(e.g.,* AE288) is inserted at amino acid 745 corresponding to mature full length FVIII, with additional a2 region in between FVIII and Fc (A1-a1-A2-a2-288XTEN-a3-A3-C1-C2-a2-Fc). VWF057 is a VWF-Fc fusion construct that comprises D'D3 domain of the VWF protein (with two amino acid substitutions in D'D3 domain, *i.e.,* C336A and C379A) linked to the Fc region via a VWF linker, which comprises LVPRG thrombin site ("LVPRG"; SEQ ID NO: 6) and GS linker ("GS"), wherein an XTEN sequence (*i.e.,* a 144XTEN) is inserted between D'D3 domain and the VWF linker (D'D3-144XTEN-GS+LVPRG-Fc). VWF059 is a VWF-Fc fusion construct that comprises D'D3 domain of the VWF protein (with two amino acid substitutions in D'D3 domain, *i.e.,* C336A and C379A) linked to the Fc region via an acidic region 2 (a2) of FVIII as a VWF linker, wherein an XTEN sequence *(i.e.,* a 144XTEN) is inserted between D'D3 domain and the VWF linker. VWF062 is a VWF-Fc fusion construct that comprises D'D3 domain of the VWF protein (with two amino acid substitutions in D'D3 domain, *i.e.,* C336A and C379A) linked to the Fc region, wherein an XTEN sequence *(e.g.,* a 144XTEN) is inserted between D'D3 domain and the Fc region (D'D3-144XTEN-Fc).

Figure 10 shows a schematic diagram representing FVIII/VWF heterodimer constructs, for example, FVIII169/VWF057, FVIII169/VWF059, FVIII 169/VWF059A, and FVIII169/VWF073. The arrow shows the site where an optional linker is added to introduce a thrombin cleavage site. FVIII169/VWF057 has a linker comprising LVPRG (SEQ ID NO: 6). FVIII169/VWF059 has a linker comprising the FVIII a2 region *(i.e.,* ISDKNTGDYYEDSYEDISAYLLSKNNAIEPRSFSDKTH (SEQ ID NO: 106)). FVIII169/VWF059A has a linker comprising a truncated FVIII a2 region *(i.e.,* DKNTGDYYEPSYEDISAYLLSKNNAIEPRSFSDKTH (SEQ ID NO: 88)). FVIII169/VWF073 has a linker within the VWF073 construct (SEQ ID NO: 175) comprising a fragment of the FVIII a2 region consisting of IEPRSFS (SEQ ID NO: 194).

Figures 11A-C show SDS-PAGE images following thrombin digestion of FVIII169/VWF057 and a FVIII-Fc control. Figure 11A shows staining of the SDS-PAGE gel with an anti-D3 antibody (AB 96340). Arrows highlight "LCFc:D'D3-XTEN-Fc," which is the un-cleaved, full-length FVIII169/VWF057; and "D'D3-144 XTEN," which is the resulting fragment following cleavage by thrombin. Figure 11B shows staining of the SDS-PAGE gel with an anti-HC antibody (GMA012). Arrows highlight the FVIII heavy chain ("HC") and FVIII A2 domain. Figure 11C shows the overlay of panels A and B. Samples were collected at the time points indicated at the top of each panel. Arrows point to the relevant proteins.

Figures 12A-C shows SDS-PAGE images following thrombin digestion of FVIII169/VWF059. Figure 12A shows staining of the SDS-PAGE gel with an anti-D3 antibody (AB 96340). Arrows highlight "LCFc:D'D3-XTEN-Fc," which is the un-cleaved, full-length FVIII169/VWF059; and "D'D3-144 XTEN," which is the resulting fragment following cleavage by thrombin. Figure 12B shows staining of the SDS-PAGE gel with an anti-HC antibody (GMA012). Arrows highlight the un-cleaved, full length FVIII169/VWF059; D'D3-144 XTEN-a3, which is the resulting fragment following cleavage by thrombin; and "A2," which is the A2 domain of FVIII. Figure 12C shows the overlay of panels A and B. Samples were collected at the time points indicated at the top of each panel

Figure 13 shows acute efficacy data of HemA mice treated with FVIII169/VWF059 (circle) as compared to HemA mice treated with a BDD-FVIII control (Square). Blood loss value was measured following tail clip. p = 0.9883.

DETAILED DESCRIPTION OF THE INVENTION

[0034] The present invention is directed to a chimeric protein as described in the Summary of the Invention.

I. Definitions

[0035] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a nucleotide sequence," is understood to represent one or more nucleotide sequences. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0036] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0037] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of' and/or "consisting essentially of' are also provided.

[0038] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0039] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0040] The term "about" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" can modify a numerical value above and below the stated value by a variance of, *e.g.,* 10 percent, up or down (higher or lower).

[0041] The term "polynucleotide" or "nucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct, *e.g.,* messenger RNA (mRNA) or plasmid DNA (pDNA). In certain embodiments, a polynucleotide comprises a conventional phosphodiester bond or a non-conventional bond *(e.g.,* an amide bond, such as found in peptide nucleic acids (PNA)). The term "nucleic acid" refers to any one or more nucleic acid segments, *e.g.,* DNA or RNA fragments, present in a polynucleotide. By "isolated" nucleic acid or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding a Factor VIII polypeptide contained in a vector is considered isolated for the purposes of the present invention. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) from other polynucleotides in a solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides of the present invention. Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid can include regulatory elements such as promoters, enhancers, ribosome binding sites, or transcription termination signals.

[0042] As used herein, a "coding region" or "coding sequence" is a portion of polynucleotide which consists of codons translatable into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is typically not translated into an amino acid, it may be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. The boundaries of a coding region are typically determined by a start codon at the 5' terminus, encoding the amino terminus of the resultant polypeptide, and a translation stop codon at the 3' terminus, encoding the carboxyl terminus of the resulting polypeptide. Two or more coding regions of the present invention can be present in a single polynucleotide construct, *e.g.,* on a single vector, or in separate polynucleotide constructs, *e.g.,* on separate (different) vectors. It follows, then, that a single vector can contain just a single coding region, or comprise two or more coding regions, *e.g.,* a single vector can separately encode a binding domain-A and a binding domain-B as described below. In addition, a vector, polynucleotide, or nucleic acid of the invention can encode heterologous coding regions, either fused or unfused to a nucleic acid encoding a binding domain of the invention. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

[0043] Certain proteins secreted by mammalian cells are associated with a secretory signal peptide which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that signal peptides are generally fused to the N-terminus of the polypeptide, and are cleaved from the complete or "full-length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, a native signal peptide or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, *e.g.,* a human tissue plasminogen activator (TPA) or mouse β-glucuronidase signal peptide, or a functional derivative thereof, can be used.

[0044] The term "downstream," when refers to a nucleotide sequence, means that a nucleic acid or a nucleotide sequence is located 3' to a reference nucleotide sequence. In certain embodiments, downstream nucleotide sequences relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription. The term "downstream," when refers to a polypeptide sequence, means that the amino acid or an amino acid insertion site is located at the C-terminus of the reference amino acids. For example, an insertion site immediately downstream of amino acid 745 corresponding to the mature wild type FVIII protein means that the insertion site is between amino acid 745 and amino acid 746 corresponding to the mature wild type FVIII protein.

[0045] The term "upstream" refers to a nucleotide sequence that is located 5' to a reference nucleotide sequence. In certain embodiments, upstream nucleotide sequences relate to sequences that are located on the 5' side of a coding region or starting point of transcription. For example, most promoters are located upstream of

the start site of transcription.

[0046] As used herein, the term "regulatory region" refers to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding region, and which influence the transcription, RNA processing, stability, or translation of the associated coding region. Regulatory regions may include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites and stem-loop structures. If a coding region is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0047] A polynucleotide which encodes a gene product, *e.g.,* a polypeptide, can include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. In an operable association a coding region for a gene product, *e.g.,* a polypeptide, is associated with one or more regulatory regions in such a way as to place expression of the gene product under the influence or control of the regulatory region(s). For example, a coding region and a promoter are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the gene product encoded by the coding region, and if the nature of the linkage between the promoter and the coding region does not interfere with the ability of the promoter to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can also be operably associated with a coding region to direct gene product expression.

[0048] A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (the immediate early promoter, in conjunction with intron-A), simian virus 40 (the early promoter), and retroviruses (such as Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit β-globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters *(e.g.,* promoters inducible by interferons or interleukins).

[0049] Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from picornaviruses (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

[0050] The term "expression" as used herein refers to a process by which a polynucleotide produces a gene product, for example, an RNA or a polypeptide. It includes without limitation transcription of the polynucleotide into messenger RNA (mRNA), transfer RNA (tRNA), small hairpin RNA (shRNA), small interfering RNA (siRNA) or any other RNA product, and the translation of an mRNA into a polypeptide. Expression produces a "gene product." As used herein, a gene product can be either a nucleic acid, *e.g.,* a messenger RNA produced by transcription of a gene, or a polypeptide which is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.,* polyadenylation or splicing, or polypeptides with post translational modifications, *e.g.,* methylation, glycosylation, the addition of lipids, association with other protein subunits, or proteolytic cleavage.

[0051] A "vector" refers to any vehicle for the cloning of and/or transfer of a nucleic acid into a host cell. A vector may be a replicon to which another nucleic acid segment may be attached so as to bring about the replication of the attached segment. A "replicon" refers to any genetic element (*e.g.,* plasmid, phage, cosmid, chromosome, virus) that functions as an autonomous unit of replication *in vivo, i.e.,* capable of replication under its own control. The term "vector" includes both viral and nonviral vehicles for introducing the nucleic acid into a cell *in vitro, ex vivo* or *in vivo.* A large number of vectors are known and used in the art including, for example, plasmids,

modified eukaryotic viruses, or modified bacterial viruses. Insertion of a polynucleotide into a suitable vector can be accomplished by ligating the appropriate polynucleotide fragments into a chosen vector that has complementary cohesive termini.

[0052] Vectors may be engineered to encode selectable markers or reporters that provide for the selection or identification of cells that have incorporated the vector. Expression of selectable markers or reporters allows identification and/or selection of host cells that incorporate and express other coding regions contained on the vector. Examples of selectable marker genes known and used in the art include: genes providing resistance to ampicillin, streptomycin, gentamycin, kanamycin, hygromycin, bialaphos herbicide, sulfonamide, and the like; and genes that are used as phenotypic markers, *i.e.,* anthocyanin regulatory genes, isopentanyl transferase gene, and the like. Examples of reporters known and used in the art include: luciferase (Luc), green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), -galactosidase (LacZ), -glucuronidase (Gus), and the like. Selectable markers may also be considered to be reporters.

[0053] The term "plasmid" refers to an extra-chromosomal element often carrying a gene that is not part of the central metabolism of the cell, and usually in the form of circular double-stranded DNA molecules. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear, circular, or supercoiled, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell.

[0054] Eukaryotic viral vectors that can be used include, but are not limited to, adenovirus vectors, retrovirus vectors, adeno-associated virus vectors, and poxvirus, *e.g.,* vaccinia virus vectors, baculovirus vectors, or herpesvirus vectors. Non-viral vectors include plasmids, liposomes, electrically charged lipids (cytofectins), DNA-protein complexes, and biopolymers.

[0055] A "cloning vector" refers to a "replicon," which is a unit length of a nucleic acid that replicates sequentially and which comprises an origin of replication, such as a plasmid, phage or cosmid, to which another nucleic acid segment may be attached so as to bring about the replication of the attached segment. Certain cloning vectors are capable of replication in one cell type, *e.g.,* bacteria and expression in another, *e.g.,* eukaryotic cells. Cloning vectors typically comprise one or more sequences that can be used for selection of cells comprising the vector and/or one or more multiple cloning sites for insertion of nucleic acid sequences of interest.

[0056] The term "expression vector" refers to a vehicle designed to enable the expression of an inserted nucleic acid sequence following insertion into a host cell. The inserted nucleic acid sequence is placed in operable association with regulatory regions as described above.

[0057] Vectors are introduced into host cells by methods well known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector transporter.

[0058] "Culture," "to culture" and "culturing," as used herein, means to incubate cells under *in vitro* conditions that allow for cell growth or division or to maintain cells in a living state. "Cultured cells," as used herein, means cells that are propagated *in vitro.*

[0059] As used herein, the term "polypeptide" is intended to encompass a singular "polypeptide" as well as plural "polypeptides," and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein," "amino acid chain," or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of "polypeptide," and the term "polypeptide" can be used instead of, or

interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide can be derived from a natural biological source or produced recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It can be generated in any manner, including by chemical synthesis.

[0060] An "isolated" polypeptide or a fragment, variant, or derivative thereof refers to a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can simply be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for the purpose of the invention, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

[0061] Also disclosed are fragments or variants of polypeptides, and any combination thereof. The term "fragment" or "variant" when referring to polypeptide binding domains or binding molecules of the present invention include any polypeptides which retain at least some of the properties (e.g., FcRn binding affinity for an FcRn binding domain or Fc variant, coagulation activity for an FVIII variant, or FVIII binding activity for the VWF fragment) of the reference polypeptide. Fragments of polypeptides include proteolytic fragments, as well as deletion fragments, in addition to specific antibody fragments discussed elsewhere herein, but do not include the naturally occurring full-length polypeptide (or mature polypeptide). Variants of polypeptide binding domains or binding molecules of the present invention include fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. Variants can be naturally or non-naturally occurring. Non-naturally occurring variants can be produced using art-known mutagenesis techniques. Variant polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions or additions.

[0062] The term "VWF protein" or "VWF proteins" used herein means any VWF fragments that interact with FVIII and retain at least one or more properties that are normally provided to FVIII by full-length VWF, e.g., preventing premature activation to FVIIIa, preventing premature proteolysis, preventing association with phospholipid membranes that could lead to premature clearance, preventing binding to FVIII clearance receptors that can bind naked FVIII but not VWF-bound FVIII, and/or stabilizing the FVIII heavy chain and light chain interactions.

[0063] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (*e.g.,* lysine, arginine, histidine), acidic side chains (*e.g.,* aspartic acid, glutamic acid), uncharged polar side chains *(e.g.,* glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.,* alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.,* threonine, valine, isoleucine) and aromatic side chains (*e.g.,* tyrosine, phenylalanine, tryptophan, histidine). Thus, if an amino acid in a polypeptide is replaced with another amino acid from the same side chain family, the substitution is considered to be conservative. In another embodiment, a string of amino acids can be conservatively replaced with a structurally similar string that differs in order and/or composition of side chain family members.

[0064] As known in the art, "sequence identity" between two polypeptides is determined by comparing the amino acid sequence of one polypeptide to the sequence of a second polypeptide. When discussed herein, whether any particular polypeptide is at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to another polypeptide can be determined using methods and computer programs/software known in the art such as, but not limited to, the BESTFIT program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). BESTFIT uses the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981), to find the best segment of homology between two sequences. When using BESTFIT or any other sequence

alignment program to determine whether a particular sequence is, for example, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full-length of the reference polypeptide sequence and that gaps in homology of up to 5% of the total number of amino acids in the reference sequence are allowed.

[0065] As used herein, an "amino acid corresponding to" or an "equivalent amino acid" in a VWF sequence or a FVIII protein sequence is identified by alignment to maximize the identity or similarity between a first VWF or FVIII sequence and a second VWF or FVIII sequence. The number used to identify an equivalent amino acid in a second VWF or FVIII sequence is based on the number used to identify the corresponding amino acid in the first VWF or FVIII sequence.

[0066] As used herein, the term "insertion site" refers to a position in a FVIII polypeptide, or fragment, variant, or derivative thereof, which is immediately upstream of the position at which a heterologous moiety can be inserted. An "insertion site" is specified as a number, the number being the number of the amino acid in mature native FVIII (SEQ ID NO: 65) to which the insertion site corresponds, which is immediately N-terminal to the position of the insertion. For example, the phrase "a3 comprises an XTEN at an insertion site which corresponds to amino acid 1656 of SEQ ID NO: 65" indicates that the heterologous moiety is located between two amino acids corresponding to amino acid 1656 and amino acid 1657 of SEQ ID NO: 65.

[0067] The phrase "immediately downstream of an amino acid" as used herein refers to position right next to the terminal carboxyl group of the amino acid. Similarly, the phrase "immediately upstream of an amino acid" refers to the position right next to the terminal amine group of the amino acid. Therefore, the phrase "between two amino acids of an insertion site" as used herein refers to a position in which an XTEN or any other polypeptide is inserted between two adjacent amino acids. Thus, the phrases "inserted immediately downstream of an amino acid" and "inserted between two amino acids of an insertion site" are used synonymously with "inserted at an insertion site."

[0068] The terms "inserted," "is inserted," "inserted into" or grammatically related terms, as used herein refers to the position of an XTEN in a chimeric polypeptide relative to the analogous position in native mature human FVIII. As used herein the terms refer to the characteristics of the recombinant FVIII polypeptide relative to native mature human FVIII, and do not indicate, imply or infer any methods or process by which the chimeric polypeptide was made. For example, in reference to a chimeric polypeptide provided herein, the phrase "an XTEN is inserted into immediately downstream of residue 745 of the FVIII polypeptide" means that the chimeric polypeptide comprises an XTEN immediately downstream of an amino acid which corresponds to amino acid 745 in native mature human FVIII, *e.g.,* bounded by amino acids corresponding to amino acids 745 and 746 of native mature human FVIII.

[0069] A "fusion" or "chimeric" protein comprises a first amino acid sequence linked to a second amino acid sequence with which it is not naturally linked in nature. The amino acid sequences which normally exist in separate proteins can be brought together in the fusion polypeptide, or the amino acid sequences which normally exist in the same protein can be placed in a new arrangement in the fusion polypeptide, *e.g.,* fusion of a Factor VIII domain of the invention with an Ig Fc domain. A fusion protein is created, for example, by chemical synthesis, or by creating and translating a polynucleotide in which the peptide regions are encoded in the desired relationship. A chimeric protein can further comprises a second amino acid sequence associated with the first amino acid sequence by a covalent, non-peptide bond or a non-covalent bond.

[0070] As used herein, the term "half-life" refers to a biological half-life of a particular polypeptide *in vivo.* Half-life may be represented by the time required for half the quantity administered to a subject to be cleared from the circulation and/or other tissues in the animal. When a clearance curve of a given polypeptide is constructed as a function of time, the curve is usually biphasic with a rapid α-phase and longer β-phase. The α-phase typically represents an equilibration of the administered Fc polypeptide between the intra- and extra-vascular space and is, in part, determined by the size of the polypeptide. The β-phase typically represents the catabolism of the

polypeptide in the intravascular space. In some embodiments, FVIII and chimeric proteins comprising FVIII are monophasic, and thus do not have an alpha phase, but just the single beta phase. Therefore, in certain embodiments, the term half-life as used herein refers to the half-life of the polypeptide in the β-phase. The typical β phase half-life of a human antibody in humans is 21 days.

[0071] The term "linked" as used herein refers to a first amino acid sequence or nucleotide sequence covalently or non-covalently joined to a second amino acid sequence or nucleotide sequence, respectively. The first amino acid or nucleotide sequence can be directly joined or juxtaposed to the second amino acid or nucleotide sequence or alternatively an intervening sequence can covalently join the first sequence to the second sequence. The term "linked" means not only a fusion of a first amino acid sequence to a second amino acid sequence at the C-terminus or the N-terminus, but also includes insertion of the whole first amino acid sequence (or the second amino acid sequence) into any two amino acids in the second amino acid sequence (or the first amino acid sequence, respectively). In one embodiment, the first amino acid sequence can be linked to a second amino acid sequence by a peptide bond or a linker. The first nucleotide sequence can be linked to a second nucleotide sequence by a phosphodiester bond or a linker. The linker can be a peptide or a polypeptide (for polypeptide chains) or a nucleotide or a nucleotide chain (for nucleotide chains) or any chemical moiety (for both polypeptide and polynucleotide chains). The term "linked" is also indicated by a hyphen (-).

[0072] As used herein the term "associated with" refers to a covalent or non-covalent bond formed between a first amino acid chain and a second amino acid chain. In one embodiment, the term "associated with" means a covalent, non-peptide bond or a non-covalent bond. This association can be indicated by a colon, *i.e.,* (:). In another embodiment, it means a covalent bond except a peptide bond. For example, the amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a thiol group on a second cysteine residue. In most naturally occurring IgG molecules, the CH1 and CL regions are associated by a disulfide bond and the two heavy chains are associated by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system). Examples of covalent bonds include, but are not limited to, a peptide bond, a metal bond, a hydrogen bond, a disulfide bond, a sigma bond, a pi bond, a delta bond, a glycosidic bond, an agnostic bond, a bent bond, a dipolar bond, a Pi backbond, a double bond, a triple bond, a quadruple bond, a quintuple bond, a sextuple bond, conjugation, hyperconjugation, aromaticity, hapticity, or antibonding. Non-limiting examples of non-covalent bond include an ionic bond (*e.g.,* cation-pi bond or salt bond), a metal bond, an hydrogen bond (*e.g.,* dihydrogen bond, dihydrogen complex, low-barrier hydrogen bond, or symmetric hydrogen bond), van der Walls force, London dispersion force, a mechanical bond, a halogen bond, aurophilicity, intercalation, stacking, entropic force, or chemical polarity.

[0073] The term "monomer-dimer hybrid" used herein refers to a chimeric protein comprising a first polypeptide chain and a second polypeptide chain, which are associated with each other by a disulfide bond, wherein the first chain comprises a clotting factor, *e.g.,* Factor VIII, and a first Fc region and the second chain comprises, consists essentially of, or consists of a second Fc region without the clotting factor. The monomer-dimer hybrid construct thus is a hybrid comprising a monomer aspect having only one clotting factor and a dimer aspect having two Fc regions.

[0074] As used herein, the term "cleavage site" or "enzymatic cleavage site" refers to a site recognized by an enzyme. Certain enzymatic cleavage sites comprise an intracellular processing site. In one embodiment, a polypeptide has an enzymatic cleavage site cleaved by an enzyme that is activated during the clotting cascade, such that cleavage of such sites occurs at the site of clot formation. Exemplary such sites include, *e.g.,* those recognized by thrombin, Factor XIa or Factor Xa. Exemplary FXIa cleavage sites include, *e.g.,* TQSFNDFTR (SEQ ID NO: 1) and SVSQTSKLTR (SEQ ID NO: 3). Exemplary thrombin cleavage sites include, *e.g.,* DFLAEGGGVR (SEQ ID NO: 4), TTKIKPR (SEQ ID NO: 5), LVPRG (SEQ ID NO: 6), ALRPR (SEQ ID NO: 7), ISDKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 106), DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88), and IEPRSFS (SEQ ID NO: 194). Other enzymatic cleavage sites are known in the art and described in elsewhere herein.

[0075] As used herein, the term "processing site" or "intracellular processing site" refers to a type of enzymatic cleavage site in a polypeptide which is a target for enzymes that function after translation of the polypeptide. In one embodiment, such enzymes function during transport from the Golgi lumen to the trans-Golgi compartment. Intracellular processing enzymes cleave polypeptides prior to secretion of the protein from the cell. Examples of such processing sites include, *e.g.,* those targeted by the PACE/furin (where PACE is an acronym for Paired basic Amino acid Cleaving Enzyme) family of endopeptidases. These enzymes are localized to the Golgi membrane and cleave proteins on the carboxyterminal side of the sequence motif Arg-[any residue]-(Lys or Arg)-Arg. As used herein the "furin" family of enzymes includes, *e.g.,* PCSK1 (also known as PC1/Pc3), PCSK2 (also known as PC2), PCSK3 (also known as furin or PACE), PCSK4 (also known as PC4), PCSK5 (also known as PC5 or PC6), PCSK6 (also known as PACE4), or PCSK7 (also known as PC7/LPC, PC8, or SPC7). Other processing sites are known in the art.

[0076] In constructs that include more than one processing or cleavage site, it will be understood that such sites may be the same or different.

[0077] The term "Furin" refers to the enzymes corresponding to EC No. 3.4.21.75. Furin is subtilisin-like proprotein convertase, which is also known as PACE (Paired basic Amino acid Cleaving Enzyme). Furin deletes sections of inactive precursor proteins to convert them into biologically active proteins. During its intracellular transport, pro-peptide of VWF can be cleaved from mature VWF molecule by a Furin enzyme. In some embodiments, Furin cleaves the D1D2 from the D'D3 of VWF. In other embodiments, a nucleotide sequence encoding Furin can be expressed together with the nucleotide sequence encoding a VWF fragment so that D1D2 domains can be cleaved off intracellularly by Furin.

[0078] In constructs that include more than one processing or cleavage site, it will be understood that such sites may be the same or different.

[0079] A "processable linker" as used herein refers to a linker comprising at least one intracellular processing site, which are described elsewhere herein.

[0080] Hemostatic disorder, as used herein, means a genetically inherited or acquired condition characterized by a tendency to hemorrhage, either spontaneously or as a result of trauma, due to an impaired ability or inability to form a fibrin clot. Examples of such disorders include the hemophilias. The three main forms are hemophilia A (factor VIII deficiency), hemophilia B (factor IX deficiency or "Christmas disease") and hemophilia C (factor XI deficiency, mild bleeding tendency). Other hemostatic disorders include, *e.g.,* Von Willebrand disease, Factor XI deficiency (PTA deficiency), Factor XII deficiency, deficiencies or structural abnormalities in fibrinogen, prothrombin, Factor V, Factor VII, Factor X or factor XIII, Bernard-Soulier syndrome, which is a defect or deficiency in GPIb. GPIb, the receptor for VWF, can be defective and lead to lack of primary clot formation (primary hemostasis) and increased bleeding tendency), and thrombasthenia of Glanzman and Naegeli (Glanzmann thrombasthenia). In liver failure (acute and chronic forms), there is insufficient production of coagulation factors by the liver; this may increase bleeding risk.

[0081] The chimeric molecules of the invention can be used prophylactically. As used herein the term "prophylactic treatment" refers to the administration of a molecule prior to a bleeding episode. In one embodiment, the subject in need of a general hemostatic agent is undergoing, or is about to undergo, surgery. The chimeric protein of the invention can be administered prior to or after surgery as a prophylactic. The chimeric protein of the invention can be administered during or after surgery to control an acute bleeding episode. The surgery can include, but is not limited to, liver transplantation, liver resection, dental procedures, or stem cell transplantation.

[0082] The chimeric protein of the invention is also used for on-demand treatment. The term "on-demand treatment" refers to the administration of a chimeric molecule in response to symptoms of a bleeding episode or before an activity that may cause bleeding. In one aspect, the on-demand treatment can be given to a subject

when bleeding starts, such as after an injury, or when bleeding is expected, such as before surgery. In another aspect, the on-demand treatment can be given prior to activities that increase the risk of bleeding, such as contact sports.

[0083] As used herein the term "acute bleeding" refers to a bleeding episode regardless of the underlying cause. For example, a subject may have trauma, uremia, a hereditary bleeding disorder (*e.g.,* factor VII deficiency) a platelet disorder, or resistance owing to the development of antibodies to clotting factors.

[0084] Treat, treatment, treating, as used herein refers to, *e.g.,* the reduction in severity of a disease or condition; the reduction in the duration of a disease course; the amelioration of one or more symptoms associated with a disease or condition; the provision of beneficial effects to a subject with a disease or condition, without necessarily curing the disease or condition, or the prophylaxis of one or more symptoms associated with a disease or condition. In one embodiment, the term "treating" or "treatment" means maintaining a FVIII trough level at least about 1 IU/dL, 2 IU/dL, 3 IU/dL, 4 IU/dL, 5 IU/dL, 6 IU/dL, 7 IU/dL, 8 IU/dL, 9 IU/dL, 10 IU/dL, 11 IU/dL, 12 IU/dL, 13 IU/dL, 14 IU/dL, 15 IU/dL, 16 IU/dL, 17 IU/dL, 18 IU/dL, 19 IU/dL, or 20 IU/dL in a subject by administering a chimeric protein of the invention. In another embodiment, treating or treatment means maintaining a FVIII trough level between about 1 and about 20 IU/dL, about 2 and about 20 IU/dL, about 3 and about 20 IU/dL, about 4 and about 20 IU/dL, about 5 and about 20 IU/dL, about 6 and about 20 IU/dL, about 7 and about 20 IU/dL, about 8 and about 20 IU/dL, about 9 and about 20 IU/dL, or about 10 and about 20 IU/dL. Treatment or treating of a disease or condition can also include maintaining FVIII activity in a subject at a level comparable to at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20% of the FVIII activity in a non-hemophiliac subject. The minimum trough level required for treatment can be measured by one or more known methods and can be adjusted (increased or decreased) for each person.

II. Chimeric Proteins

[0085] The present disclosure is directed to extending a half-life of a chimeric protein using a VWF protein fused to an XTEN sequence by preventing or inhibiting a FVIII half-life limiting factor, *i.e.,* endogenous VWF, from associating with the FVIII protein. Endogenous VWF associates with about 95% to about 98% of FVIII in non-covalent complexes. While endogenous VWF is a FVIII half-life limiting factor, endogenous VWF bound to a FVIII protein is also known to protect FVIII in various ways. For example, full length VWF (as a multimer having about 250 kDa) can protect FVIII from protease cleavage and FVIII activation, stabilize the FVIII heavy chain and/or light chain, and prevent clearance of FVIII by scavenger receptors. But, at the same time, endogenous VWF limits the FVIII half-life by preventing pinocytosis and by clearing FVIII-VWF complex from the system through the VWF clearance pathway. It is believed, while not bound by a theory, that endogenous VWF is a half-life limiting factor that prevents the half-life of a chimeric protein fused to a half-life extender from being longer than about two-fold that of wild-type FVIII. Therefore, the present disclosure is directed to preventing or inhibiting interaction between endogenous VWF and a FVIII protein using a VWF protein comprising a D' domain and a D3 domain (e.g., a VWF fragment) and at the same time to increasing a half-life of resulting FVIII protein(s) by using an XTEN sequence in combination with an Ig constant region or a portion thereof. In particular, the present invention shows that a shorter XTEN sequence (*i.e.,* XTEN that contains less than 288 amino acids in length, *i.e.,* XTEN that is shorter than 288 amino acids) is better in extending a half-life of the chimeric protein.

[0086] In one embodiment, the invention is directed to a chimeric protein as described in the Summary of the Invention, wherein the second polypeptide, which comprises a VWF fragment comprising a D' domain and a D3 domain of VWF fused to a second Fc region by an XTEN sequence in-between, comprising an XTEN sequence containing less than 288 amino acid residues and wherein the first polypeptide is linked to or associated with the second polypeptide. In other embodiments, the chimeric protein exhibits a longer half-life compared to a corresponding fusion protein comprising the first polypeptide and the second polypeptide, wherein the second polypeptide comprises an XTEN sequence containing at least 288 amino acids, *e.g.,* AE288, *e.g.,* SEQ ID NO: 8.

In still other embodiments, the XTEN sequence in the second polypeptide contains at least 144 amino acids, but less than 288 amino acids.

[0087] The chimeric protein of the invention can further comprise a second XTEN sequence which links the FVIII protein with the first Fc region.

[0088] In certain embodiments, the invention is directed to a chimeric protein comprising (i) a first polypeptide which comprises a FVIII protein fused to a first Fc region and (ii) a second polypeptide which comprises a VWF fragment comprising a D' domain and a D3 domain of VWF fused to a second Fc region by a first XTEN sequence in-between, wherein the XTEN sequence contains less than 288 amino acid residues and wherein the first polypeptide is linked to or associated with the second polypeptide, and wherein the first polypeptide further comprises a second XTEN sequence which is inserted at one or more insertion sites within the FVIII protein

[0089] The second and/or third XTEN sequences can be any length of XTEN amino acids. For example, the second and/or third XTEN sequences are disclosed elsewhere herein, *e.g.,* AE42, AE72, AE864, AE576, AE288, AE144, AG864, AG576, AG288, and AG144, e.g., SEQ ID NO: 8; SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 17; SEQ ID NO: 54; SEQ ID NO: 19; SEQ ID NO: 16; SEQ ID NO: 18; SEQ ID NO: 15; SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 14; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; or SEQ ID NO: 63. In a particular embodiment, the second and/or third XTEN sequence is AE288 or AG288, *e.g.,* SEQ ID NO: 8 or 19.

[0090] In certain embodiments, the FVIII protein is fused to the first Fc region by an optional linker, wherein an optional XTEN sequence (X2) is inserted at one or more insertion sites within the FVIII protein or is fused to the FVIII protein or to the first Fc region thereof, and (ii) a second polypeptide which comprises a VWF fragment comprising a D' domain and a D3 domain of VWF fused to a second Fc region by an XTEN sequence (X1) between the VWF fragment and the second Fc region, wherein the XTEN sequence (X1) contains less than 288 amino acid residues and wherein the first polypeptide and the second polypeptide are associated. In some embodiments, the FVIII protein is fused to the first Fc region by an optional linker, wherein an optional XTEN sequence (X2) is inserted at one or more insertion sites within the FVIII protein or is fused to the FVIII protein or to the first Fc region, and (ii) the VWF fragment is fused to the second Fc region by an XTEN sequence (X1) between the VWF fragment and the second Fc region, wherein the XTEN sequence (X1) contains less than 288 amino acid residues and is fused to the second Fc region by the cleavable linker and wherein the first polypeptide and the second polypeptide are associated. In a particular embodiment, the cleavable linker is a thrombin cleavable linker.

[0091] In some embodiments, the chimeric protein of the invention is two polypeptide chains, the first chain comprising the first polypeptide described above and the second chain comprising the second polypeptide described above. For example, the two polypeptide chains comprise (i) a first chain comprising a single chain FVIII protein, a first Fc region, and an XTEN sequence which is inserted at one or more insertion sites within the FVIII protein , and (ii) a second chain comprising the VWF fragment fused to the second Fc region by the XTEN sequence (X1) in-between, wherein the XTEN sequence (X1) contains less than 288 amino acids.

[0092] In certain embodiments, the chimeric protein of the invention is two polypeptide chains, a first chain comprising a heavy chain of a FVIII protein and a second chain comprising, from N-terminus to C-terminus, a light chain of a FVIII protein, the XTEN sequence which is inserted at one or more insertion sites within the FVIII protein or is fused to the FVIII protein or to the first Fc region, and the first Fc region, an optional linker (e.g., a processable linker), the VWF fragment, the XTEN sequence (X1), the cleavable linker, and the second Fc region.

II.A. Von Willebrand Factor (VWF) Proteins

[0093] VWF (also known as F8VWF) is a large multimeric glycoprotein present in blood plasma and produced constitutively in endothelium (in the Weibel-Palade bodies), megakaryocytes (a-granules of platelets), and subendothelian connective tissue. The basic VWF monomer is a 2813 amino acid protein. Every monomer contains a number of specific domains with a specific function, the D'/D3 domain (which binds to Factor VIII), the A1 domain (which binds to platelet GPIb-receptor, heparin, and/or possibly collagen), the A3 domain (which binds to collagen), the C1 domain (in which the RGD domain binds to platelet integrin αIIbβ3 when this is activated), and the "cysteine knot" domain at the C-terminal end of the protein (which VWF shares with platelet-derived growth factor (PDGF), transforming growth factor-β (TGFβ) and β-human chorionic gonadotropin (βHCG)).

[0094] In this disclosure, the VWF protein may be a VWF fragment. The term "a VWF fragment" as used herein includes, but is not limited to, functional VWF fragments comprising a D' domain and a D3 domain, which are capable of inhibiting binding of endogenous VWF to FVIII. In one embodiment, the VWF fragment binds to the FVIII protein. In another embodiment, the VWF fragment blocks the VWF binding site on the FVIII protein, thereby inhibiting interaction of the FVIII protein with endogenous VWF. The VWF fragments include derivatives, variants, mutants, or analogues that retain these activities of VWF.

[0095] The 2813 monomer amino acid sequence for human VWF is reported as Accession Number _NP_000543.2_ in Genbank. The nucleotide sequence encoding the human VWF is reported as Accession Number _NM_000552.3_ in Genbank. A nucleotide sequence of human VWF is designated as SEQ ID NO: 20. SEQ ID NO: 21 is the amino acid sequence of full-length VWF. Each domain of VWF is listed in Table 1.

TABLE 1. VWF Sequences

VWF domains	Amino acid Sequence
VWF Signal Peptide (Amino acids 1 to 22 of SEQ ID NO: 21)	1 MIPARFAGVL LALALILPGT LC 22
VWF D1D2 region (Amino acids 23 to 763 of SEQ ID NO: 21)	23 AEGTRGRS STARCSLFGS DFVNTFDGSM 51 YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG 101 TVTQGDQRVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL 151 SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC 201 ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC 251 EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME 301 YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTECPC 351 VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD 401 NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG 451 LHNSLVKLKH GAGVAMDGQD IQLPLLKGDL RIQHTVTASV RLSYGEDLQM 501 DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG 551 NAWKLHGDCQ DLQKQHSDPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
	601 PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCEL 651 NCPKGQVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP PGLYMDERGD 701 CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD 751 AVLSSPLSHR SKR 763
VWF D' Domain	764 SLSCRPP MVKLVCPADN LRAEGLECTK TCQNYDLECM 801 SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE

VWF domains	Amino acid Sequence
	TVKIGCNTCV 851 CRDRKWNCTD HVCDAT 866
VWF D3 Domain	867 CSTI GMAHYLTFDG LKYLFPGECQ YVLVQDYCGS 901 NPGTFRILVG NKGCSHPSVK CKKRVTILVE GGEIELFDGE VNVKRPMKDE 951 THFEVVESGR YIILLLGKAL SVVWDRHLSI SVVLKQTYQE KVCGLCGNFD 1001 GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI 1051 MKQTMVDSSC RILTSDVFQD CNKLVDPEPY LDVCIYDTCS CESIGDCACF 1101 CDTIAAYAHV CAQHGKVVTW RTATLCPQSC EERNLRENGY ECEWRYNSCA 1151 PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE 1201 VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP 1240
VWF A1 Domain	1241 GGLVVPPTDA 1251 PVSPTTLYVE DISEPPLHDF YCSRLLDLVF LLDGSSRLSE AEFEVLKAFV 1301 VDMMERLRIS QKWVRVAVVE YHDGSHAYIG LKDRKRPSEL RRIASQVKYA 1351 GSQVASTSEV LKYTLFQIFS KIDRPEASRI ALLLMASQEP QRMSRNFVRY 1401 VQGLKKKKVI VIPVGIGPHA NLKQIRLIEK QAPENKAFVL SSVDELEQQR 1451 DEIVSYLCDL APEAPPPTLP PDMAQVTVG 1479
	1480 P GLLGVSTLGP KRNSMVLDVA 1501 FVLEGSDKIG EADFNRSKEF MEEVIQRMDV GQDSIHVTVL QYSYMVTVEY 1551 PFSEAQSKGD ILQRVREIRY QGGNRTNTGL ALRYLSDHSF LVSQGDREQA 1600 1601 PNLVYMVTGN PASDEIKRLP GDIQVVPIGV GPNANVQELE RIGWPNAPIL 1651 IQDFETLPRE APDLVLQRCC SGEGLQIPTL SPAPDCSQPL DVILLLDGSS 1701 SFPASYFDEM KSFAKAFISK ANIGPRLTQV SVLQYGSITT IDVPWNVVPE 1751 KAHLLSLVDV MQREGGPSQI GDALGFAVRY LTSEMHGARP GASKAVVILV 1801 TDVSVDSVDA AADAARSNRV TVFPIGIGDR YDAAQLRILA GPAGDSNVVK 1851 LQRIEDLPTM VTLGNSFLHK LCSGFVRICM DEDGNEKRPG DVWTLPDQCH
	1901 TVTCQPDGQT LLKSHRVNCD RGLRPSCPNS QSPVKVEETC GCRWTCPCVC 1951 TGSSTRHIVT FDGQNFKLTG SCSYVLFQNK EQDLEVILHN GACSPGARQG 2001 CMKSIEVKHS ALSVEXHSDM EVTVNGRLVS VPYVGGNMEV NVYGAIMHEV 2051 RFNHLGHIFT FTPQNNEFQL QLSPKTFASK TYGLCGICDE NGANDFMLRD 2101 GTVTTDWKTL VQEWTVQRPG QTCQPILEEQ CLVPDSSHCQ VLLLPLFAEC 2151 HKVLAPATFY AICQQDSCHQ EQVCEVIASY AHLCRTNGVC VDWRTPDFCA 2201 MSCPPSLVYN HCEHGCPRHC DGNVSSCGDH PSEGCFCPPD KVMLEGSCVP 2251 EEACTQCIGE DGVQHQFLEA WVPDHQPCQI CTCLSGRKVN CTTQPCPTAK 2301 APTCGLCEVA RLRQNADQCC PEYECVCDPV SCDLPPVPHC ERGLQPTLTN 2351 PGECRPNFTC ACRKEECKRV SPPSCPPHRL PTLRKTQCCD EYECACNCVN 2401 STVSCPLGYL ASTATNDCGC TTTTCLPDKV CVHRSTIYPV GQFWEEGCDV 2451 CTCTDMEDAV MGLRVAQCSQ KPCEDSCRSG FTYVLHEGEC

VWF domains	Amino acid Sequence
	CGRCLPSACE 2501 VVTGSPRGDS QSSWKSVGSQ WASPENPCLI NECVRVKEEV FIQQRNVSCP 2551 QLEVPVCPSG FQLSCKTSAC CPSCRCERME ACMLNGTVIG PGKTVMIDVC 2601 TTCRCMVQVG VISGFKLECR KTTCNPCPLG YKEENNTGEC CGRCLPTACT 2651 IQLRGGQIMT LKRDETLQDG CDTHFCKVNE RGEYFWEKRV TGCPPFDEHK 2701 CLAEGGKIMK IPGTCCDTCE EPECNDITAR LQYVKVGSCK SEVEVDIHYC 2751 QGKCASKAMY SIDINDVQDQ CSCCSPTRTE PMQVALHCTN GSVVYHEVLN 2801 AMECKCSPRK CSK
	Nucleotide Sequence (SEQ ID NO: 20)
Full-length VWF	1 ATGATTCCTG CCAGATTTGC CGGGGTGCTG CTTGCTCTGG CCCTCATTTT 51 GCCAGGGACC CTTTGTGCAG AAGGAACTCG CGGCAGGTCA TCCACGGCCC 101 GATGCAGCCT TTTCGGAAGT GACTTCGTCA ACACCTTTGA TGGGAGCATG 151 TACAGCTTTG CGGGATACTG CAGTTACCTC CTGGCAGGGG GCTGCCAGAA 201 ACGCTCCTTC TCGATTATTG GGGACTTCCA GAATGGCAAG AGAGTGAGCC 251 TCTCCGTGTA TCTTGGGGAA TTTTTTGACA TCCATTTGTT TGTCAATGGT 301 ACCGTGACAC AGGGGGACCA AAGAGTCTCC ATGCCCTATG CCTCCAAAGG 351 GCTGTATCTA GAAACTGAGG CTGGGTACTA CAAGCTGTCC GGTGAGGCCT 401 ATGGCTTTGT GGCCAGGATC GATGGCAGCG GCAACTTTCA AGTCCTGCTG 451 TCAGACAGAT ACTTCAACAA GACCTGCGGG CTGTGTGGCA ACTTTAACAT 501 CTTTGCTGAA GATGACTTTA TGACCCAAGA AGGGACCTTG ACCTCGGACC
	551 CTTATGACTT TGCCAACTCA TGGGCTCTGA GCAGTGGAGA ACAGTGGTGT 601 GAACGGGCAT CTCCTCCCAG CAGCTCATGC AACATCTCCT CTGGGGAAAT 651 GCAGAAGGGC CTGTGGGAGC AGTGCCAGCT TCTGAAGAGC ACCTCGGTGT 701 TTGCCCGCTG CCACCCTCTG GTGGACCCCG AGCCTTTTGT GGCCCTGTGT 751 GAGAAGACTT TGTGTGAGTG TGCTGGGGGG CTGGAGTGCG CCTGCCCTGC 801 CCTCCTGGAG TACGCCCGGA CCTGTGCCCA GGAGGGAATG GTGCTGTACG 851 GCTGGACCGA CCACAGCGCG TGCAGCCCAG TGTGCCCTGC TGGTATGGAG 901 TATAGGCAGT GTGTGTCCCC TTGCGCCAGG ACCTGCCAGA GCCTGCACAT 951 CAATGAAATG TGTCAGGAGC GATGCGTGGA TGGCTGCAGC TGCCCTGAGG 1001 GACAGCTCCT GGATGAAGGC CTCTGCGTGG AGAGCACCGA GTGTCCCTGC 1051 GTGCATTCCG GAAAGCGCTA CCCTCCCGGC ACCTCCCTCT CTCGAGACTG 1101 CAACACCTGC ATTTGCCGAA ACAGCCAGTG GATCTGCAGC AATGAAGAAT 1151 GTCCAGGGGA GTGCCTTGTC ACTGGTCAAT CCCACTTCAA GAGCTTTGAC 1201 AACAGATACT TCACCTTCAG TGGGATCTGC CAGTACCTGC TGGCCCGGGA 1251 TTGCCAGGAC CACTCCTTCT CCATTGTCAT TGAGACTGTC CAGTGTGCTG 1301 ATGACCGCGA CGCTGTGTGC ACCCGCTCCG TCACCGTCCG GCTGCCTGGC 1351 CTGCACAACA GCCTTGTGAA ACTGAAGCAT GGGGCAGGAG TTGCCATGGA 1401 TGGCCAGGAC ATCCAGCTCC CCCTCCTGAA AGGTGACCTC CGCATCCAGC 1451 ATACAGTGAC GGCCTCCGTG CGCCTCAGCT ACGGGGAGGA CCTGCAGATG 1501 GACTGGGATG GCCGCGGGAG GCTGCTGGTG AAGCTGTCCC CCGTCTATGC 1551 CGGGAAGACC TGCGGCCTGT GTGGGAATTA CAATGGCAAC CAGGGCGACG 1601 ACTTCCTTAC CCCCTCTGGG CTGGCRGAGC CCCGGGTGGA GGACTTCGGG 1651 AACGCCTGGA AGCTGCACGG GGACTGCCAG GACCTGCAGA AGCAGCACAG 1701 CGATCCCTGC GCCCTCAACC CGCGCATGAC CAGGTTCTCC

Nucleotide Sequence (SEQ ID NO: 20)

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     GAGGAGGCGT
1751 GCGCGGTCCT GACGTCCCCC ACATTCGAGG CCTGCCATCG
     TGCCGTCAGC
1801 CCGCTCCCCT ACCTCCCCAA CTGCCCCTAC GACGTGTCCT
     CCTGCTCGGA
1851 CGGCCGCGAG TGCCTGTGCG GCGCCCTGGC CAGCTATGCC
     GCGGCCTGCG
1901 CGGGGAGAGG CGTGCGCGTC GCGTGGCGCG AGCCAGGCCG
     CTGTGAGCTG
1951 AACTGCCCGA AAGGCCAGGT GTACCTGCAG TGCGGGACCC
     CCTGCAACCT
2001 GACCTGCCGC TCTCTCTCTT ACCCGGATGA GGAATGCAAT
     GAGGCCTGCC
2051 TGGAGGGCTG CTTCTGCCCC CCAGGGCTCT ACATGGATGA
     GAGGGGGGAC
2101 TGCGTGCCCA AGGCCCAGTG CCCCTGTTAC TATGACGGTG
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2151 GCCAGAAGAC ATCTTCTCAG ACCATCACAC CATGTGCTAC
     TGTGAGGATG
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2201 GCTTCATGCA CTGTACCATG AGTGGAGTCC CCGGAAGCTT
     GCTGCCTGAC
2251 GCTGTCCTCA GCAGTCCCCT GTCTCATCGC AGCAAAAGGA
     GCCTATCCTG
2301 TCGGCCCCCC ATGGTCAAGC TGGTGTGTCC CGCTGACAAC
     CTGCGGGCTG
2351 AAGGGCTCGA GTGTACCAAA ACGTGCCAGA ACTATGACCT
     GGAGTGCATG
2401 AGCATGGGCT GTGTCTCTGG CTGCCTCTGC CCCCCGGGCA
     TGGTCCGGCA
2451 TGAGAACAGA TGTGTGGCCC TGGAAAGGTG TCCCTGCTTC
     CATCAGGGCA
2501 AGGAGTATGC CCCTGGAGAA ACAGTGAAGA TTGGCTGCAA
     CACTTGTGTC
2551 TGTCGGGACC GGAACTGGAA CTGCACAGAC CATGTGTGTG
     ATGCCACGTG
2601 CTCCACGATC GGCATGGCCC ACTACCTCAC CTTCGACGGG
     CTCAAATACC
2651 TGTTCCCCGG GGAGTGCCAG TACGTTCTGG TGCAGGATTA
     CTGCGGCAGT
2701 AACCCTGGGA CCTTTCGGAT CCTAGTGGGG AATAAGGGAT
     GCAGCCACCC
2751 CTCAGTGAAA TGCAAGAAAC GGGTCACCAT CCTGGTGGAG
     GGAGGAGAGA
2801 TTGAGCTGTT TGACGGGGAG GTGAATGTGA AGAGGCCCAT
     GAAGGATGAG
2851 ACTCACTTTG AGGTGGTGGA GTCTGGCCGG TACATCATTC
     TGCTGCTGGG
2901 CAAAGCCCTC TCCGTGGTCT GGGACCGCCA CCTGAGCATC
     TCCGTGGTCC
2951 TGAAGCAGAC ATACCAGGAG AAAGTGTGTG GCCTGTGTGG
     GAATTTTGAT
3001 GGCATCCAGA ACAATGACCT CACCAGCAGC AACCTCCAAG
     TGGAGGAAGA
3051 CCCTGTGGAC TTTGCGAACT CCTGGAAAGT GAGCTCGCAG
     TGTGCTGACA
3101 CCAGAAAAGT GCCTCTGGAC TCATCCCCTG CCACCTGCCA
     TAACAACATC
3151 ATGAAGCAGA CGATGGTGGA TTCCTCCTGT AGAATCCTTA
     CCAGTGACGT
3201 CTTCCAGGAC TGCAACAAGC TGGTGGACCC CGAGCCATAT
     CTGGATGTCT
3251 GCATTTACGA CACCTGCTCC TGTGAGTCCA TTGGGGACTG
     CGCCTGCTTC
3301 TGCGACACCA TTGCTGCCTA TGCCCACGTG TGTGCCCAGC
     ATGGCAAGGT
3351 GGTGACCTGG AGGACGGCCA CATTGTGCCC CCAGAGCTGC
     GAGGAGAGGA
3401 ATCTCCGGGA GAACGGGTAT GAGTGTGAGT GGCGCTATAA
     CAGCTGTGCA
3451 CCTGCCTGTC AAGTCACGTG TCAGCACCCT GAGCCACTGG
     CCTGCCCTGT
3501 GCAGTGTGTG GAGGCCTGCC ATGCCCACTG CCCTCCAGGG
     AAAATCCTGG
3551 ATGAGCTTTT GCAGACCTGC GTTGACCCTG AAGACTGTCC
     AGTGTGTGAG
3601 GTGGCTGGCC GGCGTTTTGC CTCAGGAAAG AAAGTCAGCT
     TGAATCCCAG
3651 TGACCCTGAG CACTGCCAGA TTTGCCACTG TGATGTTGTC
     AACCTCACCT
3701 GTGAAGCCTG CCAGGAGCCG GGAGGCCTGG TGGTGCCTCC
     CACAGATGCC
3751 CCGGTGAGCC CCACCACTCT GTATGTGGAG GACATCTCGG
     AACCGCCGTT
3801 GCACGATTTC TACTGCAGCA GGCTACTGGA CCTGGTCTTC
     CTGCTGGATG
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3851 GCTCCTCCAG GCTGTCCGAG GCTGAGTTTG AAGTGCTGAA
     GGCCTTTGTG
3901 GTGGACATGA TGGAGCGGCT GCGCATCTCC CAGAAGTGGG
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Nucleotide Sequence (SEQ ID NO: 20)

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3951  CGTGGTGGAG TACCACGACG GCTCCCACGC CTACATCGGG
      CTCAAGGACC
4001  GGAAGCGACC GTCAGAGCTG CGGCGCATTG CCAGCCAGGT
      GAAGTATGCG
4051  GGCAGCCAGG TGGCCTCCAC CAGCGAGGTC TTGAAATACA
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4101  AATCTTCAGC AAGATCGACC GCCCTGAAGC CTCCCGCATC
      GCCCTGCTCC
4151  TGATGGCCAG CCAGGAGCCC CAACGGATGT CCCGGAACTT
      TGTCCGCTAC
4201  GTCCAGGGCC TGAAGAAGAA GAAGGTCATT GTGATCCCGG
      TGGGCATTGG
4251  GCCCCATGCC AACCTCAAGC AGATCCGCCT CATCGAGAAG
      CAGGCCCCTG
4301  AGAACAAGGC CTTCGTGCTG AGCAGTGTGG ATGAGCTGGA
      GCAGCAAAGG
4351  GACGAGATCG TTAGCTACCT CTGTGACCTT GCCCCTGAAG
      CCCCTCCTCC
4401  TACTCTGCCC CCCGACATGG CACAAGTCAC TGTGGGCCCG
      GGGCTCTTGG
4451  GGGTTTCGAC CCTGGGGCCC AAGAGGAACT CCATGGTTCT
      GGATGTGGCG
4501  TTCGTCCTGG AAGGATCGGA CAAAATTGGT GAAGCCGACT
      TCAACAGGAG
4551  CAAGGAGTTC ATGGAGGAGG TGATTCAGCG GATGGATGTG
      GGCCAGGACA
4601  GCATCCACGT CACGGTGCTG CAGTACTCCT ACATGGTGAC
      CGTGGAGTAC
4651  CCCTTCAGCG AGGCACAGTC CAAAGGGGAC ATCCTGCAGC
      GGGTGCGAGA
4701  GATCCGCTAC CAGGGCGGCA ACAGGACCAA CACTGGGCTG
      GCCCTGCGGT
4751  ACCTCTCTGA CCACAGCTTC TTGGTCAGCC AGGGTGACCG
      GGAGCAGGCG
4801  CCCAACCTGG TCTACATGGT CACCGGAAAT CCTGCCTCTG
      ATGAGATCAA
4851  GAGGCTGCCT GGAGACATCC AGGTGGTGCC CATTGGAGTG
      GGCCCTAATG
4901  CCAACGTGCA GGAGCTGGAG AGGATTGGCT GGCCCAATGC
      CCCTATCCTC
4951  ATCCAGGACT TTGAGACGCT CCCCCGAGAG GCTCCTGACC
      TGGTGCTGCA
5001  GAGGTGCTGC TCCGGAGAGG GGCTGCAGAT CCCCACCCTC
      TCCCCTGCAC
5051  CTGACTGCAG CCAGCCCCTG GACGTGATCC TTCTCCTGGA
      TGGCTCCTCC
5101  AGTTTCCCAG CTTCTTATTT TGATGAAATG AAGAGTTTCG
      CCAAGGCTTT
5151  CATTTCAAAA GCCAATATAG GGCCTCGTCT CACTCAGGTG
      TCAGTGCTGC
5201  AGTATGGAAG CATCACCACC ATTGACGTGC CATGGAACGT
      GGTCCCGGAG
5251  AAAGCCCACT TGCTGAGCCT TGTGGACGTC ATGCAGCGGG
      AGGGAGGCCC
5301  CAGCCAAATC GGGGATGCCT TGGGCTTTGC TGTGCGATAC
      TTGACTTCAG
5351  AAATGCATGG TGCCAGGCCG GGAGCCTCAA AGGCGGTGGT
      CATCCTGGTC
5401  ACGGACGTCT CTGTGGATTC AGTGGATGCA GCAGCTGATG
      CCGCCAGGTC
5451  CAACAGAGTG ACAGTGTTCC CTATTGGAAT TGGAGATCGC
      TACGATGCAG


5501  CCCAGCTACG GATCTTGGCA GGCCCAGCAG GCGACTCCAA
      CGTGGTGAAG
5551  CTCCAGCGAA TCGAAGACCT CCCTACCATG GTCACCTTGG
      GCAATTCCTT
5601  CCTCCACAAA CTGTGCTCTG GATTTGTTAG GATTTGCATG
      GATGAGGATG
5651  GGAATGAGAA GAGGCCCGGG GACGTCTGGA CCTTGCCAGA
      CCAGTGCCAC
5701  ACCGTGACTT GCCAGCCAGA TGGCCAGACC TTGCTGAAGA
      GTCATCGGGT
5751  CAACTGTGAC CGGGGGCTGA GGCCTTCGTG CCCTAACAGC
      CAGTCCCCTG
5801  TTAAAGTGGA AGAGACCTGT GGCTGCCGCT GGACCTGCCC
      CTCYGTGTCC
5851  ACAGGCAGCT CCACTCGGCA CATCGTGACC TTTGATGGGC
      AGAATTTCAA
5901  GCTGACTGGC AGCTGTTCTT ATGTCCTATT TCAAAACAAG
      GAGCAGGACC
5951  TGGAGGTGAT TCTCCATAAT GGTGCCTGCA GCCCTGGAGC
      AAGGCAGGGC
6001  TGCATGAAAT CCATCGAGGT CAAGCACACT GCCCTCTCCC
      TCGAGSTGCA
6051  CAGTGACATG GAGGTGACGG TGAATGGGAG ACTGGTCTCT
      GTTCCTTACG
6101  TGGGTGGGAA CATGGAAGTC AACGTTTATG GTGCCATCAT
      GCATGAGGTC
6151  AGATTCAATC ACCTTGGTCA CATCTTCACA TTCACTCCAC
      AAAACAATCA
6201  GTTCCAACTG CAGCTCAGCC CCAAGACTTT TGCTTCAAAG
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Nucleotide Sequence (SEQ ID NO: 20)

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6251 TGTGTGGGAT CTGTGATGAG AACGGAGCCA ATGACTTCAT
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6301 GGCACAGTCA CCACAGACTG GAAAACACTT GTTCAGGAAT
     GGACTGTGCA
6351 GCGGCCAGGG CAGACGTGCC AGCCCATCCT GGAGGAGCAG
     TGTCTTGTCC
6401 CCGACAGCTC CCACTGCCAG GTCCTCCTCT TACCACTGTT
     TGCTGAATGC
6451 CACAAGGTCC TGGCTCCAGC CACATTCTAT GCCATCTGCC
     AGCAGGACAG
6501 TTGCCACCAG GAGCAAGTGT GTGAGGTGAT CGCCTCTTAT
     GCCCACCTCT
6551 GTCGGACCAA CGGGGTCTGC GTTGACTGGA GGACACCTGA
     TTTCTGTGCT
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     ATGGCTGTCC
6651 CCGGCACTGT GATGGCAACG TGAGCTCCTG TGGGGACCAT
     CCCTCCGAAG
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     CTGTGTCCCT
6751 GAAGAGGCCT GCACTCAGTG CATTGGTGAG GATGGAGTCC
     AGCACCAGCT
6801 CCTGGAAGCC TGGGTCCCGG ACCACCAGCC CTGTCAGATC
     TGCACATGCC
6851 TCAGCGGGCG GAAGGTCAAC TGCACAACGC AGCCCTGCCC
     CACGGCCAAA
6901 GCTCCCACGT GTGGCCTGTG TGAAGTAGCC CGCCTCCGCC
     AGAATGCAGA
6951 CCAGTGCTGC CCCGAGTATG AGTGTGTGTG TGACCCAGTG
     AGCTGTGACC
7001 TGCCCCCAGT GCCTCACTGT GAACGTGGCC TCCAGCCCAC
     ACTGACCAAC
7051 CCTGGCGAGT GCAGACCCAA CTTCACCTGC GCCTGCAGGA
     AGGAGGAGTG
7101 CAAAAGAGTG TCCCCACCCT CCTGCCCCCC GCACCGTTTG
     CCCACCCTTC


7151 GGAAGACCCA GTGCTGTGAT GAGTATGAGT GTGCCTGCAA
     CTGTGTCAAC
7201 TCCACAGTGA GCTGTCCCCT TGGGTACTTG GCCTCAACCG
     CCACCAATGA
7251 CTGTGGCTGT ACCACAACCA CCTGCCTTCC CGACAAGGTG
     TGTGTCCACC
7301 GAAGCACCAT CTACCCTGTG GGCCAGTTCT GGGAGGAGGG
     CTGCGATGTG
7351 TGCACCTGCA CCGACATGGA GGATGCCGTG ATGGGCCTCC
     GCGTGGCCCA
7401 GTGCTCCCAG AAGCCCTGTG AGGACAGCTG TCGGTCGGGC
     TTCACTTACG
7451 TTCTGCATGA AGGCGAGTGC TGTGGAAGGT GCCTGCCATC
     TGCCTGTGAG
7501 GTGGTGACTG GCTCACCGCG GGGGGACTCC CAGTCTTCCT
     GGAAGAGTGT
7551 CGGCTCCCAG TGGGCCTCCC CGGAGAACCC CTGCCTCATC
     AATGAGTGTG
7601 TCCGAGTGAA GGAGGAGGTC TTTATACAAC AAAGGAACGT
     CTCCTGCCCC
7651 CAGCTGGAGG TCCCTGTCTG CCCCTCGGGC TTTCAGCTGA
     GCTGTAAGAC
7701 CTCAGCGTGC TGCCCAAGCT GTCGCTGTGA GCGCATGGAG
     GCCTGCATGC
7751 TCAATGGCAC TGTCATTGGG CCCGGGAAGA CTGTGATGAT
     CGATGTGTGC
7801 ACGACCTGCC GCTGCATGGT GCAGGTGGGG GTCATCTCTG
     GATTCAAGCT
7851 GGAGTGCAGG AAGACCACCT GCAACCCCTG CCCCCTGGGT
     TACAAGGAAG
7901 AAAATAACAC AGGTGAATGT TGTGGGAGAT GTTTGCCTAC
     GGCTTGCACC
7951 ATTCAGCTAA GAGGAGGACA GATCATGACA CTGAAGCGTG
     ATGAGACGCT
8001 CCAGGATGGC TGTGATACTC ACTTCTGCAA GGTCAATGAG
     AGAGGAGAGT
8051 ACTTCTGGGA GAAGAGGGTC ACAGGCTGCC CACCCTTTGA
     TGAACACAAG
8101 TGTCTTGCTG AGGGAGGTAA AATTATGAAA ATTCCAGGCA
     CCTGCTGTGA
8151 CACATGTGAG GAGCCTGAGT GCAACGACAT CACTGCCAGG
     CTGCAGTATG
8201 TCAAGGTGGG AAGCTGTAAG TCTGAAGTAG AGGTGGATAT
     CCACTACTGC
8251 CAGGGCAAAT GTGCCAGCAA AGCCATGTAC TCCATTGACA
     TCAACGATGT
8301 GCAGGACCAG TGCTCCTGCT GCTCTCCGAC ACGGACGGAG
     CCCATGCAGG
8351 TGGCCCTGCA CTGCACCAAT GGCTCTGTTG TGTACCATGA
     GGTTCTCAAT
8401   GCCATGGAGT GCAAATGCTC CCCCAGGAAG TGCAGCAAGT GA
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	Nucleotide Sequence (SEQ ID NO: 20)

[0096] The VWF fragment as used herein comprises a D' domain and a D3 domain of VWF, wherein the VWF fragment binds to Factor VIII (FVIII) and inhibits binding of endogenous VWF (full-length VWF) to FVIII. The VWF fragment comprising the D' domain and the D3 domain can further comprise a VWF domain selected from the group consisting of an A1 domain, an A2 domain, an A3 domain, a D1 domain, a D2 domain, a D4 domain, a B1 domain, a B2 domain, a B3 domain, a C1 domain, a C2 domain, a CK domain, one or more fragments thereof, and any combinations thereof. In one embodiment, a VWF fragment comprises, consists essentially of, or consists of: (1) the D' and D3 domains of VWF or fragments thereof; (2) the D1, D', and D3 domains of VWF or fragments thereof; (3) the D2, D', and D3 domains of VWF or fragments thereof; (4) the D1, D2, D', and D3 domains of VWF or fragments thereof; or (5) the D1, D2, D', D3, and A1 domains of VWF or fragments thereof. The VWF fragment described herein does not contain a site binding to a VWF clearance receptor. The VWF fragment of the present invention can comprise any other sequences linked to or fused to the VWF fragment. For example, a VWF fragment described herein can further comprise a signal peptide.

[0097] In one embodiment, the VWF fragment comprising a D' domain and a D3 domain binds to or is associated with a FVIII protein. By binding to or associating with a FVIII protein, a VWF fragment of the invention protects FVIII from protease cleavage and FVIII activation, stabilizes the heavy chain and light chain of FVIII, and prevents clearance of FVIII by scavenger receptors. In another embodiment, the VWF fragment binds to or associates with a FVIII protein and blocks or prevents binding of the FVIII protein to phospholipid and activated Protein C. By preventing or inhibiting binding of the FVIII protein with endogenous, full-length VWF, the VWF fragment of the invention reduces the clearance of FVIII by VWF clearance receptors and thus extends half-life of the chimeric protein. The half-life extension of a chimeric protein is thus due to the binding of or associating with the VWF fragment lacking a VWF clearance receptor binding site to the FVIII protein and shielding or protecting of the FVIII protein by the VWF fragment from endogenous VWF which contains the VWF clearance receptor binding site. The FVIII protein bound to or protected by the VWF fragment can also allow recycling of a FVIII protein. By eliminating the VWF clearance pathway receptor binding sites contained in the full length VWF molecule, the FVIII/VWF heterodimers of the invention are shielded from the VWF clearance pathway, further extending FVIII half-life.

[0098] As disclosed herein, a VWF protein may comprise a D' domain and a D3 domain of VWF, wherein the D' domain is at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 866 of SEQ ID NO: 21, wherein the VWF protein prevents or inhibits binding of endogenous VWF to FVIII. A VWF protein may comprise the D' domain and the D3 domain of VWF, wherein the D3 domain is at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 867 to 1240 of SEQ ID NO: 21, wherein the VWF protein prevents or inhibits binding of endogenous VWF to FVIII. A VWF protein disclosed herein may comprise, consist essentially of, or consist of the D' domain and D3 domain of VWF, which are at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 1240 of SEQ ID NO: 21, wherein the VWF protein prevents or inhibits binding of endogenous VWF to FVIII. A VWF protein may comprise, consist essentially of, or consist of the D1, D2, D', and D3 domains at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23 to 1240 of SEQ ID NO: 21, wherein the VWF protein prevents or inhibits binding of endogenous VWF to FVIII. A VWF protein may further comprise a signal peptide operably linked thereto.

[0099] In some embodiments, a VWF fragment useful for the invention consists essentially of or consists of (1) the D'D3 domain, the D1D'D3 domain, D2D'D3 domain, or D1D2D'D3 domain and (2) an additional VWF sequence up to about 10 amino acids (*e.g.,* any sequences from amino acids 764 to 1240 of SEQ ID NO: 21 to amino acids 764 to 1250 of SEQ ID NO: 21), up to about 15 amino acids (*e.g.,* any sequences from amino acids 764 to 1240 of SEQ ID NO: 21 to amino acids 764 to 1255 of SEQ ID NO: 21), up to about 20 amino acids (e.g., any sequences from amino acids 764 to 1240 of SEQ ID NO: 21 to amino acids 764 to 1260 of SEQ ID NO: 21), up to about 25 amino acids *(e.g.,* any sequences from amino acids 764 to 1240 of SEQ ID NO: 21 to amino

acids 764 to 1265 of SEQ ID NO: 21), or up to about 30 amino acids *(e.g.,* any sequences from amino acids 764 to 1240 of SEQ ID NO: 21 to amino acids 764 to 1260 of SEQ ID NO: 21). In a particular embodiment, the VWF fragment comprising or consisting essentially of the D' domain and the D3 domain is not the full-length mature VWF. In some embodiments, the D1D2 domain is expressed in trans with the D'D3 domain. In some embodiments, the D1D2 domain is expressed in cis with the D'D3 domain.

[0100] In other embodiments, the VWF fragment comprising the D'D3 domains linked to the D1D2 domains further comprises an intracellular cleavage site, *e.g.,* (a cleavage site by PACE (furin) or PC5), allowing cleavage of the D1D2 domains from the D'D3 domains upon expression. Non-limiting examples of the intracellular cleavage site are disclosed elsewhere herein.

[0101] In yet other embodiments, the VWF fragment comprises a D' domain and a D3 domain, but does not comprise an amino acid sequence selected from the group consisting of (1) amino acids 1241 to 2813 corresponding to SEQ ID NO: 21, (2) amino acids 1270 to amino acids 2813 corresponding to SEQ ID NO: 21, (3) amino acids 1271 to amino acids 2813 corresponding to SEQ ID NO: 21, (4) amino acids 1272 to amino acids 2813 corresponding to SEQ ID NO: 21, (5) amino acids 1273 to amino acids 2813 corresponding to SEQ ID NO: 21, (6) amino acids 1274 to amino acids 2813 corresponding to SEQ ID NO: 21, and any combinations thereof.

[0102] In some embodiments, a VWF fragment of the invention comprises a D' domain and a D3 domain, but does not comprise at least one VWF domain selected from the group consisting of (1) an A1 domain, (2) an A2 domain, (3) an A3 domain, (4) a D4 domain, (5) a B1 domain, (6) a B2 domain, (7) a B3 domain, (8) a C1 domain, (9) a C2 domain, (10) a CK domain, (11) a CK domain and C2 domain, (12) a CK domain, a C2 domain, and a C1 domain, (13) a CK domain, a C2 domain, a C1 domain, a B3 domain, (14) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, (15) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, and a B1 domain, (16) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, and a D4 domain, (17) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, a D4 domain, and an A3 domain, (18) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, a D4 domain, an A3 domain, and an A2 domain, (19) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, a D4 domain, an A3 domain, an A2 domain, and an A1 domain, and (20) any combinations thereof.

[0103] In yet other embodiments, the VWF fragment comprises the D'D3 domains and one or more domains or modules. Examples of such domains or modules include, but are not limited to, the domains and modules disclosed in Zhour et al., Blood published online April 6, 2012: DOI 10.1182/blood-2012-01-405134. For example, the VWF fragment can comprise the D'D3 domain and one or more domains or modules selected from the group consisting of A1 domain, A2 domain, A3 domain, D4N module, VWD4 module, C8-4 module, TIL-4 module, C1 module, C2 module, C3 module, C4 module, C5 module, C5 module, C6 module, and any combinations thereof.

[0104] In still other embodiments, the VWF fragment is linked to a heterologous moiety, wherein the heterologous moiety is linked to the N-terminus or the C-terminus of the VWF fragment. The heterologous moiety can be a half-life extender.

[0105] In certain embodiments, the VWF fragment is a monomer having only one VWF fragment. In some embodiments, the VWF fragment of the present invention can have one or more amino acid substitutions, deletions, additions, or modifications. In one embodiment, the VWF fragment can include amino acid substitutions, deletions, additions, or modifications such that the VWF fragment is not capable of forming a disulfide bond or forming a dimer or a multimer. In another embodiment, the amino acid substitution is within the D' domain and the D3 domain. In a particular embodiment, a VWF fragment useful for the invention contains at least one amino acid substitution at a residue corresponding to residue 1099, residue 1142, or both residues 1099 and 1142 corresponding to SEQ ID NO: 21. The at least one amino acid substitution can be any amino

acids that are not occurring naturally in the wild type VWF. For example, the amino acid substitution can be any amino acids other than cysteine, e.g., Isoleucine, Alanine, Leucine, Asparagine, Lysine, Aspartic acid, Methionine, Phenylalanine, Glutamic acid, Threonine, Glutamine, Tryptophan, Glycine, Valine, Proline, Serine, Tyrosine, Arginine, or Histidine. In a particular embodiment, the VWF fragment contains an alanine residue substituted for the residues corresponding to residues 1099 and 1142 of SEQ ID NO: 21. In another example, the amino acid substitution has one or more amino acids that prevent or inhibit the VWF fragments from forming multimers.

[0106] In certain embodiments, the VWF fragment useful herein can be further modified to improve its interaction with FVIII, *e.g.,* to improve binding affinity to FVIII. As a non-limiting example, the VWF fragment comprises a serine residue at the residue corresponding to amino acid 764 of SEQ ID NO: 21 and a lysine residue at the residue corresponding to amino acid 773 of SEQ ID NO: 21. Residues 764 and/or 773 can contribute to the binding affinity of the VWF fragments to FVIII. In other embodiments, The VWF fragments useful for the invention can have other modifications, *e.g.,* the protein can be pegylated, glycosylated, hesylated, or polysialylated.

II. B. XTEN Sequences

[0107] As used herein "XTEN sequence" refers to extended length polypeptides with non-naturally occurring, substantially non-repetitive sequences that are composed mainly of small hydrophilic amino acids, with the sequence having a low degree or no secondary or tertiary structure under physiologic conditions. As a chimeric protein partner, XTENs can serve as a carrier, conferring certain desirable pharmacokinetic, physicochemical and pharmaceutical properties when linked to a VWF fragment or a FVIII sequence of the invention to create a chimeric protein. Such desirable properties include but are not limited to enhanced pharmacokinetic parameters and solubility characteristics. As used herein, "XTEN" specifically excludes antibodies or antibody fragments such as single-chain antibodies or Fc fragments of a light chain or a heavy chain.

[0108] The present invention provides that a shorter XTEN sequence provides an improved half-life extending property compared to a longer XTEN sequence when the XTEN sequence is fused to the VWF fragment and/or the second Fc region. Therefore, the XTEN sequence fused to the VWF fragment and/or the second Fc region contains less than 288 amino acids in length, i.e., is shorter than 288 amino acids. In another embodiment, the XTEN sequence fused to the VWF protein and/or the second Fc region comprises at least 144 amino acids, but less than 288 amino acids. In other embodiments, the XTEN sequence fused to a VWF fragment and/or the second Fc region.The chimeric protein of the invention can further comprise an additional (second, third, or more) XTEN sequences. The additional XTEN sequence can further be fused to the FVIII protein or the first Fc region. The additional XTEN sequences can be any length. For example, the additional XTEN sequence fused to the FVIII protein or the first Fc region is a peptide or a polypeptide having greater than about 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1200, 1400, 1600, 1800, or 2000 amino acid residues. In certain embodiments, the additional XTEN sequence is a peptide or a polypeptide having greater than about 20 to about 3000 amino acid residues, greater than about 30 to about 2500 residues, greater than about 40 to about 2000 residues, greater than about 50 to about 1500 residues, greater than about 60 to about 1000 residues, greater than about 70 to about 900 residues, greater than about 80 to about 800 residues, greater than about 90 to about 700 residues, greater than about 100 to about 600 residues, greater than about 110 to about 500 residues, or greater than about 120 to about 400 residues.

[0109] The XTEN sequences can comprise one or more sequence motif of 9 to 14 amino acid residues or an amino acid sequence at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the sequence motif, wherein the motif comprises, consists essentially of, or consists of 4 to 6 types of amino acids selected from the group consisting of glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P). *See* US 2010-0239554 A1.

[0110] The XTEN sequence may comprise non-overlapping sequence motifs in which at least about 80%, or at least about 85%, 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% of the sequence consists of multiple units of non-overlapping sequences selected from a single motif family selected from Table 2A, resulting in a family sequence. As used herein, "family" means that the XTEN has motifs selected only from a single motif category from Table 2A; *i.e.,* AD, AE, AF, AG, AM, AQ, BC, or BD XTEN, and that any other amino acids in the XTEN not from a family motif are selected to achieve a needed property, such as to permit incorporation of a restriction site by the encoding nucleotides, incorporation of a cleavage sequence, or to achieve a better linkage to FVIII or VWF. In XTEN families, an XTEN sequence may comprise multiple units of non-overlapping sequence motifs of the AD motif family, or of the AE motif family, or of the AF motif family, or of the AG motif family, or of the AM motif family, or of the AQ motif family, or of the BC family, or of the BD family, with the resulting XTEN exhibiting the range of homology described above. The XTEN may comprise multiple units of motif sequences from two or more of the motif families of Table 2A. These sequences can be selected to achieve desired physical/chemical characteristics, including such properties as net charge, hydrophilicity, lack of secondary structure, or lack of repetitiveness that are conferred by the amino acid composition of the motifs, described more fully below. The motifs incorporated into the XTEN can be selected and assembled using the methods described herein to achieve an XTEN of about 36 to about 3000 amino acid residues.

Table 2A. XTEN Sequence Motifs of 12 Amino Acids and Motif Families

Motif Family*	MOTIF SEQUENCE
AD	GESPGGSSGSES (SEQ ID NO: 24)
AD	GSEGSSGPGESS (SEQ ID NO: 25)
AD	GSSESGSSEGGP (SEQ ID NO: 26)
AD	GSGGEPSESGSS (SEQ ID NO: 27)
AE, AM	GSPAGSPTSTEE (SEQ ID NO: 28)
AE, AM, AQ	GSEPATSGSETP (SEQ ID NO: 29)
AE, AM, AQ	GTSESATPESGP (SEQ ID NO: 30)
AE, AM, AQ	GTSTEPSEGSAP (SEQ ID NO: 31)
AF, AM	GSTSESPSGTAP (SEQ ID NO: 32)
AF, AM	GTSTPESGSASP (SEQ ID NO: 33)
AF, AM	GTSPSGESSTAP (SEQ ID NO: 34)
AF, AM	GSTSSTAESPGP (SEQ ID NO: 35)
AG, AM	GTPGSGTASSSP (SEQ ID NO: 36)
AG, AM	GSSTPSGATGSP (SEQ ID NO: 37)
AG, AM	GSSPSASTGTGP (SEQ ID NO: 38)
AG, AM	GASPGTSSTGSP (SEQ ID NO: 39)
AQ	GEPAGSPTSTSE (SEQ ID NO: 40)
AQ	GTGEPSSTPASE (SEQ ID NO: 41)
AQ	GSGPSTESAPTE (SEQ ID NO: 42)
AQ	GSETPSGPSETA (SEQ ID NO: 43)
AQ	GPSETSTSEPGA (SEQ ID NO: 44)
AQ	GSPSEPTEGTSA (SEQ ID NO: 45)
BC	GSGASEPTSTEP (SEQ ID NO: 46)
BC	GSEPATSGTEPS (SEQ ID NO: 47)
BC	GTSEPSTSEPGA (SEQ ID NO: 48)

Motif Family*	MOTIF SEQUENCE
BC	GTSTEPSEPGSA (SEQ ID NO: 49)
BD	GSTAGSETSTEA (SEQ ID NO: 50)
BD	GSETATSGSETA (SEQ ID NO: 51)
BD	GTSESATSESGA (SEQ ID NO: 52)
BD	GTSTEASEGSAS (SEQ ID NO: 53)
• Denotes individual motif sequences that, when used together in various permutations, results in a "family sequence"	

[0111] In this disclosure, an XTEN sequence may be at least 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of AE42, AG42, AE48, AM48, AE72, AG72, AE108, AG108, AE144, AF144, AG144, AE180, AG180, AE216, AG216, AE252, AG252, AE288, AG288, AE324, AG324, AE360, AG360, AE396, AG396, AE432, AG432, AE468, AG468, AE504, AG504, AF504, AE540, AG540, AF540, AD576, AE576, AF576, AG576, AE612, AG612, AE624, AE648, AG648, AG684, AE720, AG720, AE756, AG756, AE792, AG792, AE828, AG828, AD836, AE864, AF864, AG864, AM875, AE912, AM923, AM1318, BC864, BD864, AE948, AE1044, AE1140, AE1236, AE1332, AE1428, AE1524, AE1620, AE1716, AE1812, AE1908, AE2004A, AG948, AG1044, AG1140, AG1236, AG1332, AG1428, AG1524, AG1620, AG1716, AG1812, AG1908, and AG2004. *See* US 2010-0239554 A1.

[0112] In this disclosure, an XTEN sequence may be at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from the group consisting of AE42 (SEQ ID NO: 9), AE72 (SEQ ID NO: 10), AE144 2A (SEQ IDNO: 55), AE144_3B (SEQ ID NO: 56), AE1444A (SEQ ID NO: 57), AE144_5A (SEQ ID NO: 58), AE144_6B (SEQ ID NO: 59), AG144_A (SEQ ID NO: 60), AG144 B (SEQ ID NO: 61), AG144_C (SEQ ID NO: 62), AG144 F (SEQ IDNO: 63), AE864 (SEQ ID NO: 15), AE576 (SEQ ID NO: 16), AE288 (SEQ ID NO: 8), AE288 2 (SEQ ID NO: 54), AE144 (SEQ ID NO: 11), AG864 (SEQ ID NO: 17), AG576 (SEQ ID NO: 18), AG288 (SEQ ID NO: 19), AG144 (SEQ ID NO: 14), and any combinations thereof. An XTEN sequence may be selected from the group consisting of AE42 (SEQ ID NO: 9), AE72 (SEQ ID NO: 10), AE144 2A (SEQ ID NO: 55), AE144_3B (SEQ ID NO: 56), AE144 4A (SEQ ID NO: 57), AE144 5A (SEQ ID NO: 58), AE144_6B (SEQ ID NO: 59), AG144_A (SEQ ID NO: 60), AG144_B (SEQ ID NO: 61), AG144_C (SEQ ID NO: 62), AG144_F (SEQ IDNO: 63), AE864 (SEQ ID NO: 15), AE576 (SEQ ID NO: 16), AE288 (SEQ ID NO: 8), AE288 2 (SEQ ID NO: 54), AE144 (SEQ ID NO: 11), AG864 (SEQ ID NO: 17), AG576 (SEQ ID NO: 18), AG288 (SEQ ID NO: 19), AG144 (SEQ ID NO: 14), and any combinations thereof. Specifically, an XTEN sequence may be AE288. The amino acid sequences for certain XTEN sequences are shown in Table 2B.

TABLE 2B. XTEN Sequences

XTEN	Amino Acid Sequence
AE42 SEQ ID NO: 9	GAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPASS
AE72 SEQ ID NO: 10	GAP TSESATPESG PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG TSTEPSEGSA PGASS
AE144 SEQ ID NO:11	GSEPATSGSETPGTSESATPESGPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEG SAPGSEPATSGSETPGSEPATSGSETPGSEPATSGSETPGTSTEPSEGSAPGTSESA PESGPGSEPATSGSETPGTSTEPSEGSAP
AE144_2A (SEQ ID NO: 55)	TSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTS TEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSTEPSEGSAPGTSTEPSEGSAPGTSES ATPESGPGTSESATPESGPG

XTEN	Amino Acid Sequence
AE144_3B (SEQ ID NO: 56)	SPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGTS TEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPG
AE144_4A (SEQ ID NO: 57)	TSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTS TEPSEGSAPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTEEGSPAGSPTSTEEGTSES ATPESGPGTSTEPSEGSAPG
AE144_5A (SEQ ID NO: 58)	TSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTS TEPSEGSAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAG SPTSTEEGSPAGSPTSTEEG
AE144_6B (SEQ ID NO: 59)	TSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESGPGSEPATSGSETPGSE PATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGSEPATSGSETPGTSES ATPESGPGTSTEPSEGSAPG
AG144 SEQ ID NO:14	GTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSST GSPGASPGTSSTGSPGSSTPSGATGSPGSSPSASTGTGPGASPGTSSTGSPGSSPSA STGTGPGTPGSGTASSSPGSSTPSGATGSP
AG144_A (SEQ ID NO: 60)	GASPGTSSTGSPGSSPSASTGTGPGSSPSASTGTGPGTPGSGTASSSPGSSTPSGATGSPGS SPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGASP GTSSTGSPGASPGTSSTGSP
AG144_B (SEQ ID NO: 61)	GTPGSGTASSSPGSSTPSGATGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGS SPSASTGTGPGSSPSASTGTGPGSSTPSGATGSPGSSTPSGATGSPGASPGTSSTGSPGASP GTSSTGSPGASPGTSSTGSP
AG144_C (SEQ ID NO: 62)	GTPGSGTASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSPSASTGTGPGT PGSGTASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATGSPGSST PSGATGSPGASPGTSSTGSP
AG144_F (SEQ ID NO: 63)	GSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATGSPGS SPSASTGTGPGASPGTSSTGSPGSSPSASTGTGPGTPGSGTASSSPGSSTPSGATGSPGSST PSGATGSPGASPGTSSTGSP
AE288 SEQ ID NO: 8	GTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESG PGTSTEPSEGSAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPES GPGSPAGSPTSTEEGSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPE SGPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSE GSAPGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAP
AE288_2 (SEQ ID NO: 54)	GSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGT STEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGSPA GSPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPAT
	SGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSPAGSPTSTEEGSPAGSPT STEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAP
AG288 SEQ ID NO:19	PGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASS SPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGATGSPGSSPSASTG

XTEN	Amino Acid Sequence
	TGPGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSPSAST GTGPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATGSPGSSPSAS TGTGPGASPGTSSTGSPGSSPSASTGTGPGTPGSGTASSSPGSSTPSGATGS
AE576 SEQ ID NO:16	GSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSA PGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTST EEGTSESATPESGPGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEG SAPGTSTEPSEGSAPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSEPATSG SETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSPAGSPTSTEEGTSTEPSEP TSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSTEP SEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEP ATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSPAGSPTSTEEGSP AGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAP
AG576 SEQ ID NO:18	PGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGSSTPSGATG SPGSSTPSGATGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTAS SSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSPSASTGTGPGTPGSGTA SSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATGSPGSSTPSG ATGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGATGSPGSSTPS GATGSPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGASPG TSSTGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSST PSGATGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGSS TPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGS STPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGS
AE864 SEQ ID NO:15	GSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSA PGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTST EEGTSESATPESGPGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEG SAPGTSTEPSEGSAPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSEPATSG SETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGSPAGSP TSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSTEP SEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEP ATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSPAGSPTSTEEGSP AGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGS EPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPG SPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEE GSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESG PGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGS APGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAP
AG864 SEQ ID NO:17	GASPGTSSTGSPGSSPSASTGTGPGSSPSASTGTGPGTPGSGTASSSPGSSTPSGATGS PGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASS SPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGASPGTSST GSPGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGSSTPSGA TGSPGSSTPSGATGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGT ASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSPSASTGTGPGTPGSG TASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATGSPGSSTP SGATGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGATGSPGSST PSGATGSPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGAS PGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGS STPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGATGSPG SSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSP GSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGS PGSSTPSGATGSPGSSPSASTGTGPGASPGTSSTGSPGSSPSASTGTGPGTPGSGTASS SPGSSTPSGATGSPGSSTPSGATGSPGASPGTSSTGSP

[0113] Where XTEN component(s) disclosed herein have less than 100% of its amino acids consisting of 4, 5, or 6 types of amino acid selected from glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P), or less than 100% of the sequence consisting of the sequence motifs from Table 3 or the XTEN sequences of Tables 4, and 13-17, the other amino acid residues of the XTEN are selected from any of the other 14 natural L-amino acids, but are preferentially selected from hydrophilic amino acids such that the XTEN sequence contains at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% hydrophilic amino acids. The XTEN amino acids that are not glycine (G), alanine (A), serine (S), threonine (T), glutamate (E)

and proline (P) are either interspersed throughout the XTEN sequence, are located within or between the sequence motifs, or are concentrated in one or more short stretches of the XTEN sequence, e.g., to create a linker between the XTEN and the FVIII or VWF components. In such cases where the XTEN component comprises amino acids other than glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P), it is preferred that less than about 2% or less than about 1% of the amino acids be hydrophobic residues such that the resulting sequences generally lack secondary structure, e.g., not having more than 2% alpha helices or 2% beta-sheets, as determined by the methods disclosed herein. Hydrophobic residues that are less favored in construction of XTEN include tryptophan, phenylalanine, tyrosine, leucine, isoleucine, valine, and methionine. Additionally, one can design the XTEN sequences to contain less than 5% or less than 4% or less than 3% or less than 2% or less than 1% or none of the following amino acids: cysteine (to avoid disulfide formation and oxidation), methionine (to avoid oxidation), asparagine and glutamine (to avoid desamidation). Thus, the XTEN component comprising other amino acids in addition to glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) may have a sequence with less than 5% of the residues contributing to alpha-helices and beta-sheets as measured by the Chou-Fasman algorithm and have at least 90%, or at least about 95% or more random coil formation as measured by the GOR algorithm.

[0114] In further embodiments, the XTEN sequence used in the invention affects the physical or chemical property, *e.g.,* pharmacokinetics, of the chimeric protein of the present invention. The XTEN sequence used in the present invention can exhibit one or more of the following advantageous properties: conformational flexibility, enhanced aqueous solubility, high degree of protease resistance, low immunogenicity, low binding to mammalian receptors, or increased hydrodynamic (or Stokes) radii. In a specific embodiment, the XTEN sequence linked to a FVIII protein in this invention increases pharmacokinetic properties such as longer terminal half-life or increased area under the curve (AUC), so that the chimeric protein described herein stays *in vivo* for an increased period of time compared to wild type FVIII. In further embodiments, the XTEN sequence used in this invention increases pharmacokinetic properties such as longer terminal half-life or increased area under the curve (AUC), so that FVIII protein stays *in vivo* for an increased period of time compared to wild type FVIII.

[0115] Disclosed herein is a FVIII/VWF fusion protein comprising a FVIII portion fused to an Fc region and a VWF portion fused to an Fc region, wherein an XTEN sequence (e.g., AE288) is inserted within the FVIII portion, and wherein an XTEN sequence having less than 288 amino acids (e.g., AE144) is inserted between the VWF portion and the Fc portion. As described in the examples, insertion of an XTEN having less than 288 amino acids between the VWF portion and the Fc portion has a greater effect on the pharmacokinetics of the chimeric protein than the insertion of an XTEN having 288 amino acids between the VWF portion and the Fc portion. For example, insertion of an XTEN sequence having less than 288 amino acids between the VWF portion and the Fc portion in FVIII/VWF fusion protein can increase the terminal half-life of the chimeric protein compared to an XTEN having 288 amino acids. In some embodiments, the terminal half-life is increased by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 30%, relative to the insertion of an XTEN sequence having 288 amino acids. In one particular embodiment, the terminal half-life is increased by at least about 35% relative to the insertion of an XTEN having 288 amino acids. Insertion of an XTEN sequence having less than 288 amino acids can also increase the AUC value of the chimeric protein. In some embodiments, AUC is increased by at least about 50%, at least about 100%, or at least about 200% relative to the insertion of an XTEN having 288 amino acids. In one particular embodiment, AUC is increased by about two-fold. Insertion of an XTEN sequence having less than 288 amino acids can also reduce the clearance of the chimeric protein. For example, clearance can be decreased by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 30%, relative to the insertion of an XTEN sequence having 288 amino acids. Insertion of an XTEN sequence having less than 288 amino acids can increase mean residence time (MRT) and/or decrease the apparent volume of distribution at steady state (Vss) relative to the insertion of an XTEN having 288 amino acids.

[0116] A variety of methods and assays can be employed to determine the physical/chemical properties of proteins comprising the XTEN sequence. Such methods include, but are not limited to analytical centrifugation, EPR, HPLC-ion exchange, HPLC-size exclusion, HPLC-reverse phase, light scattering, capillary electrophoresis,

circular dichroism, differential scanning calorimetry, fluorescence, HPLC-ion exchange, HPLC-size exclusion, IR, NMR, Raman spectroscopy, refractometry, and UV/Visible spectroscopy. Additional methods are disclosed in Amau et al., Prot Expr and Purif 48, 1-13 (2006).

[0117] Further examples of XTEN sequences are disclosed in US Patent Publication Nos. 2010/0239554 A1, 2010/0323956 A1, 2011/0046060 A1, 2011/0046061 A1, 2011/0077199 A1, or 2011/0172146 A1, or International Patent Publication Nos. WO 2010091122 A1, WO 2010144502 A2, WO 2010144508 A1, WO 2011028228 A1, WO 2011028229 A1, WO 2011028344 A2, or WO 20130122617 A1.

II.C. Factor VIII (FVIII) Protein

[0118] "A FVIII protein" as used herein means a functional FVIII polypeptide in its normal role in coagulation, unless otherwise specified. The term a FVIII protein includes a functional fragment, variant, analog, or derivative thereof that retains the function of full-length wild-type Factor VIII in the coagulation pathway. "A FVIII protein" is used interchangeably with FVIII polypeptide (or protein) or FVIII. Examples of the FVIII functions include, but not limited to, an ability to activate coagulation, an ability to act as a cofactor for factor IX, or an ability to form a tenase complex with factor IX in the presence of Ca^{2+} and phospholipids, which then converts Factor X to the activated form Xa. The FVIII protein can be the human, porcine, canine, rat, or murine FVIII protein. In addition, comparisons between FVIII from humans and other species have identified conserved residues that are likely to be required for function (Cameron et al., Thromb. Haemost. 79:317-22 (1998); US 6,251,632).

[0119] A number of tests are available to assess the function of the coagulation system: activated partial thromboplastin time (aPTT) test, chromogenic assay, ROTEM assay, prothrombin time (PT) test (also used to determine INR), fibrinogen testing (often by the Clauss method), platelet count, platelet function testing (often by PFA-100), TCT, bleeding time, mixing test (whether an abnormality corrects if the patient's plasma is mixed with normal plasma), coagulation factor assays, antiphospholipid antibodies, D-dimer, genetic tests (e.g., factor V Leiden, prothrombin mutation G20210A), dilute Russell's viper venom time (dRVVT), miscellaneous platelet function tests, thromboelastography (TEG or Sonoclot), thromboelastometry (TEM®, *e.g.,* ROTEM®), or euglobulin lysis time (ELT).

[0120] The aPTT test is a performance indicator measuring the efficacy of both the "intrinsic" (also referred to the contact activation pathway) and the common coagulation pathways. This test is commonly used to measure clotting activity of commercially available recombinant clotting factors, *e.g.,* FVIII or FIX. It is used in conjunction with prothrombin time (PT), which measures the extrinsic pathway.

[0121] ROTEM analysis provides information on the whole kinetics of haemostasis: clotting time, clot formation, clot stability and lysis. The different parameters in thromboelastometry are dependent on the activity of the plasmatic coagulation system, platelet function, fibrinolysis, or many factors which influence these interactions. This assay can provide a complete view of secondary haemostasis.

[0122] The FVIII polypeptide and polynucleotide sequences are known, as are many functional fragments, mutants and modified versions. Examples of human FVIII sequences (full-length) are shown below.

TABLE 3. Amino Acid Sequence of Full-length Factor VIII

(Full-length FVIII (FVIII signal peptide underlined; FVIII heavy chain is double underlined; B domain is italicized; and FVIII light chain is in plain text)
Signal Peptide: (SEQ ID NO: 64)
MQIELSTCFFLCLLRFCFS
Mature Factor VIII (SEQ ID NO: 65)*
ATRRYYLGAVELSWDYMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTLFVEFTDHLFNIAKPRPPWMGLL

(Full-length FVIII (FVIII signal peptide underlined; FVIII heavy chain is double underlined; B domain is italicized; and FVIII light chain is in plain text)

Signal Peptide: (SEQ ID NO: 64)

GPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFPGGSHTYVWQVLKEN
GPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKFILLFAVFDEGKSWHSETKNSL
MQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVYWHVIGMGTTPEVHSIFLEGHTFLVRNHRQASLEI
SPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVKVDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRF
DDDNSPSFIQIRSVAKKHPKTWVHYIAAEEEDWDYAPLVLAPDDRSYKSQYLNNGPQRIGRKYKKVRFMAYT
DETFKTREAIQHESGILGPLLYGEVGDTLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDFPIL
PGEIFKYKWTVTVEDGPTKSDPRCLTRYYSSFVNMERDLASGLIGPLLICYKESVDQRGNQIMSDKRNVILF
SVFDENRSWYLTENIQRFLPNPAGVQLEDPEFQASNIMHSINGYVFDSLQLSVCLHEVAYWYILSIGAQTDF
LSVFFSGYTFKHKMVYEDTLTLFPFSGETVFMSMENPGLWILGCHNSDFRNRGMTALLKVSSCDKNTGDYYE
DSYEDISAYLLSKNNAIEPRSFSQNSRHPSTRQKQFNATTIPENDIEKTDPWFAHRTPMPKIQNVSSSDLLM
LLRQSPTPHGLSLSDLQEAKYETFSDDPSPGAIDSNNSLSEMTHFRPQLHHSGDMVFTPESGLQLRLNEKLG
TTAATELKKLDFKVSSTSNNLISTIPSDNLAAGTDNTSSLGPPSMPVHYDSQLDTTLFGKKSSPLTESGGPL
SLSEENNDSKLLESGLMNSQESSWGKNVSSTESGRLFKGKRAHGPALLTKDNALFKVSISLLKTNKTSNNSA
TNRKTHIDGPSLLIENSPSVWQNILESDTEFKKVTPLIHDRMLMDKNATALRLNHMSNKTTSSKNMEMVQQK
KEGPIPPDAQNPDMSFFKMLFLPESARWIQRTHGKNSLNSGQGPSPKQLVSLGPEKSVEGQNFLSEKNKVVV
GKGEFTKDVGLKEMVFPSSRNLFLTNLDNLHENNTHNQEKKIQEEIEKKETLIQENVVLPQIHTVTGTKNFM
KNLFLLSTRQNVEGSYDGAYAPVLQDFRSLNDSTNRTKKHTAHFSKKGEEENLEGLGNQTKQIVEKYACTTR
ISPNTSQQNFVTQRSKRALKQFRLPLEETELEKRIIVDDTSTQWSKNMKHLTPSTLTQIDYNEKEKGAITQS
PLSDCLTRSHSIPQANRSPLPIAKVSSFPSIRPIYLTRVLFQDNSSHLPAASYRKKDSGVQESSHFLQGAKK
NNLSLAILTLEMTGDQREVGSLGTSATNSVTYKKVENTVLPKPDLPKTSGKVELLPKVHIYQKDLFPTETSN
GSPGHLDLVEGSLLQGTEGAIKWNEANRPGKVPFLRVATESSAKTPSKLLDPLAWDNHYGTQIPKEEWKSQE
KSPEKTAFKKKDTILSLNACESNHAIAAINEGQNKPEIEVTWAKQGRTERLCSQNPPVLKRHQREITRTTLQ
SDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQKKTRHYFIAAVERLWDYGMSSSPHVLRNRAQSGSVP
QFKKVVFQEFTDGSFTQPLYRGELNEHLGLLGPYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQRQGA
EPRKNFVKPNETKTYFWKVQHHMAPTKDEFDCKAWAYFSDVDLEKDVHSGLIGPLLVCHTNTLNPAHGRQVT
VQEFALFFTIFDETKSWYFTENMERNCRAPCNIQMEDPTFKENYRFHAINGYIMDTLPGLVMAQDQRIRWYL
LSMGSNENIHSIHFSGHVFTVRKKEEYKMALYNLYPGVFETVEMLPSKAGIWRVECLIGEHLHAGMSTLFLV
YSNKCQTPLGMASGHIRDFQITASGQYGQWAPKLARLHYSGSINAWSTKEPFSWIKVDLLAPMIIHGIKTQG
ARQKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNIFNPPIIARYIRLHPTHYSIRS
TLRMELMGCDLNSCSMPLGMESKAISDAQITASSYFTNMFATWSPSKARLHLQGRSNAWRPQVNNPKEWLQV
DFQKTMKVTGVTTQGVKSLLTSMYVKEFLISSSQDGHQWTLFFQNGKVKVFQGNQDSFTPVVNSLDPPLLTR
YLRIHPQSWVHQIALRMEVLGCEAQDLY

TABLE 4. Nucleotide Sequence Encoding Full-Length FVIII (SEQ ID NO: 66)*

661	ATG CAAATAGAGC TCTCCACCTG
721	CTTCTTTCTG TGCCTTTTGC GATTCTGCTT TAGTGCCACC AGAAGATACT ACCTGGGTGC
781	AGTGGAACTG TCATGGGACT ATATGCAAAG TGATCTCGGT GAGCTGCCTG TGGACGCAAG
841	ATTTCCTCCT AGAGTGCCAA AATCTTTTCC ATTCAACACC TCAGTCGTGT ACAAAAAGAC
901	TCTGTTTGTA GAATTCACGG ATCACCTTTT CAACATCGCT AAGCCAAGGC CACCCTGGAT
961	GGGTCTGCTA GGTCCTACCA TCCAGGCTGA GGTTTATGAT ACAGTGGTCA TTACACTTAA
1021	GAACATGGCT TCCCATCCTG TCAGTCTTCA TGCTGTTGGT GTATCCTACT GGAAAGCTTC
1081	TGAGGGAGCT GAATATGATG ATCAGACCAG TCAAAGGGAG AAAGAAGATG ATAAAGTCTT
1141	CCCTGGTGGA AGCCATACAT ATGTCTGGCA GGTCCTGAAA GAGAATGGTC CAATGGCCTC
1201	TGACCCACTG TGCCTTACCT ACTCATATCT TTCTCATGTG GACCTGGTAA AAGACTTGAA
1261	TTCAGGCCTC ATTGGAGCCC TACTAGTATG TAGAGAAGGG AGTCTGGCCA AGGAAAAGAC
1321	ACAGACCTTG CACAAATTTA TACTACTTTT TGCTGTATTT GATGAAGGGA AAAGTTGGCA
1381	CTCAGAAACA AAGAACTCCT TGATGCAGGA TAGGGATGCT GCATCTGCTC GGGCCTGGCC
1441	TAAAATGCAC ACAGTCAATG GTTATGTAAA CAGGTCTCTG CCAGGTCTGA TTGGATGCCA
1501	CAGGAAATCA GTCTATTGGC ATGTGATTGG AATGGGCACC ACTCCTGAAG TGCACTCAAT
1561	ATTCCTCGAA GGTCACACAT TTCTTGTGAG GAACCATCGC CAGGCGTCCT TGGAAATCTC
1621	GCCAATAACT TTCCTTACTG CTCAAACACT CTTGATGGAC CTTGGACAGT TTCTACTGTT

1681	TTGTCATATC TCTTCCCACC AACATGATGG CATGGAAGCT TATGTCAAAG TAGACAGCTG
1741	TCCAGAGGAA CCCCAACTAC GAATGAAAAA TAATGAAGAA GCGGAAGACT ATGATGATGA
1801	TCTTACTGAT TCTGAAATGG ATGTGGTCAG GTTTGATGAT GACAACTCTC CTTCCTTTAT
1861	CCAAATTCGC TCAGTTGCCA AGAAGCATCC TAAAACTTGG GTACATTACA TTGCTGCTGA
1921	AGAGGAGGAC TGGGACTATG CTCCCTTAGT CCTCGCCCCC GATGACAGAA GTTATAAAAG
1981	TCAATATTTG AACAATGGCC CTCAGCGGAT TGGTAGGAAG TACAAAAAAG TCCGATTTAT
2041	GGCATACACA GATGAAACCT TTAAGACTCG TGAAGCTATT CAGCATGAAT CAGGAATCTT
2101	GGGACCTTTA CTTTATGGGG AAGTTGGAGA CACACTGTTG ATTATATTTA AGAATCAAGC
2161	AAGCAGACCA TATAACATCT ACCCTCACGG AATCACTGAT GTCCGTCCTT TGTATTCAAG
2221	GAGATTACCA AAAGGTGTAA AACATTTGAA GGATTTTCCA ATTCTGCCAG GAGAAATATT
2281	CAAATATAAA TGGACAGTGA CTGTAGAAGA TGGGCCAACT AAATCAGATC CTCGGTGCCT
2341	GACCCGCTAT TACTCTAGTT TCGTTAATAT GGAGAGAGAT CTAGCTTCAG GACTCATTGG
2401	CCCTCTCCTC ATCTGCTACA AAGAATCTGT AGATCAAAGA GGAAACCAGA TAATGTCAGA
2461	CAAGAGGAAT GTCATCCTGT TTTCTGTATT TGATGAGAAC CGAAGCTGGT ACCTCACAGA
2521	GAATATACAA CGCTTTCTCC CCAATCCAGC TGGAGTGCAG CTTGAGGATC CAGAGTTCCA
2581	AGCCTCCAAC ATCATGCACA GCATCAATGG CTATGTTTTT GATAGTTTGC AGTTGTCAGT
2641	TTGTTTGCAT GAGGTGGCAT ACTGGTACAT TCTAAGCATT GGAGCACAGA CTGACTTCCT
2701	TTCTGTCTTC TTCTCTGGAT ATACCTTCAA ACACAAAATG GTCTATGAAG ACACACTCAC
2761	CCTATTCCCA TTCTCAGGAG AAACTGTCTT CATGTCGATG GAAAACCCAG GTCTATGGAT
2821	TCTGGGGTGC CACAACTCAG ACTTTCGGAA CAGAGGCATG ACCGCCTTAC TGAAGGTTTC

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2881    TAGTTGTGAC AAGAACACTG GTGATTATTA CGAGGACAGT TATGAAGATA TTTCAGCATA
2941    CTTGCTGAGT AAAAACAATG CCATTGAACC AAGAAGCTTC TCCCAGAATT CAAGACACCC
3001    TAGCACTAGG CAAAAGCAAT TTAATGCCAC CACAATTCCA GAAAATGACA TAGAGAAGAC
3061    TGACCCTTGG TTTGCACACA GAACACCTAT GCCTAAAATA CAAAATGTCT CCTCTAGTGA
3121    TTTGTTGATG CTCTTGCGAC AGAGTCCTAC TCCACATGGG CTATCCTTAT CTGATCTCCA
3181    AGAAGCCAAA TATGAGACTT TTTCTGATGA TCCATCACCT GGAGCAATAG ACAGTAATAA
3241    CAGCCTGTCT GAAATGACAC ACTTCAGGCC ACAGCTCCAT CACAGTGGGG ACATGGTATT
3301    TACCCCTGAG TCAGGCCTCC AATTAAGATT AAATGAGAAA CTGGGGACAA CTGCAGCAAC
3361    AGAGTTGAAG AAACTTGATT TCAAAGTTTC TAGTACATCA AATAATCTGA TTTCAACAAT
3421    TCCATCAGAC AATTTGGCAG CAGGTACTGA TAATACAAGT TCCTTAGGAC CCCCAAGTAT
3481    GCCAGTTCAT TATGATAGTC AATTAGATAC CACTCTATTT GGCAAAAAGT CATCTCCCCT
3541    TACTGAGTCT GGTGGACCTC TGAGCTTGAG TGAAGAAAAT AATGATTCAA AGTTGTTAGA
3601    ATCAGGTTTA ATGAATAGCC AAGAAAGTTC ATGGGGAAAA AATGTATCGT CAACAGAGAG
3661    TGGTAGGTTA TTTAAAGGGA AAAGAGCTCA TGGACCTGCT TTGTTGACTA AAGATAATGC
3721    CTTATTCAAA GTTAGCATCT CTTTGTTAAA GACAAACAAA ACTTCCAATA ATTCAGCAAC
3781    TAATAGAAAG ACTCACATTG ATGGCCCATC ATTATTAATT GAGAATAGTC CATCAGTCTG
3841    GCAAAATATA TTAGAAAGTG ACACTGAGTT TAAAAAAGTG ACACCTTTGA TTCATGACAG
3901    AATGCTTATG GACAAAAATG CTACAGCTTT GAGGCTAAAT CATATGTCAA ATAAAACTAC
3961    TTCATCAAAA AACATGGAAA TGGTCCAACA GAAAAAAGAG GGCCCCATTC CACCAGATGC
4021    ACAAAATCCA GATATGTCGT TCTTTAAGAT GCTATTCTTG CCAGAATCAG CAAGGTGGAT
4081    ACAAAGGACT CATGGAAAGA ACTCTCTGAA CTCTGGGCAA GGCCCCAGTC CAAAGCAATT
4141    AGTATCCTTA GGACCAGAAA AATCTGTGGA AGGTCAGAAT TTCTTGTCTG AGAAAAACAA
4201    AGTGGTAGTA GGAAAGGGTG AATTTACAAA GGACGTAGGA CTCAAAGAGA TGGTTTTTCC
4261    AAGCAGCAGA AACCTATTTC TTACTAACTT GGATAATTTA CATGAAAATA ATACACACAA
4321    TCAAGAAAAA AAAATTCAGG AAGAAATAGA AAAGAAGGAA ACATTAATCC AAGAGAATGT
4381    AGTTTTGCCT CAGATACATA CAGTGACTGG CACTAAGAAT TTCATGAAGA ACCTTTTCTT
4441    ACTGAGCACT AGGCAAAATG TAGAAGGTTC ATATGACGGG GCATATGCTC CAGTACTTCA
4501    AGATTTTAGG TCATTAAATG ATTCAACAAA TAGAACAAAG AAACACACAG CTCATTTCTC
4561    AAAAAAAGGG GAGGAAGAAA ACTTGGAAGG CTTGGGAAAT CAAACCAAGC AAATTGTAGA
4621    GAAATATGCA TGCACCACAA GGATATCTCC TAATACAAGC CAGCAGAATT TTGTCACGCA
4681    ACGTAGTAAG ACAGCTTTGA AACAATTCAG ACTCCCACTA GAAGAAACAG AACTTGAAAA
4741    AAGGATAATT GTGGATGACA CCTCAACCCA GTGGTCCAAA AACATGAAAC ATTTGACCCC
4801    GAGCACCCTC ACACAGATAG ACTACAATGA GAAGGAGAAA GGGGCCATTA CTCAGTCTCC
4861    CTTATCAGAT TGCCTTACGA GGAGTCATAG CATCCCTCAA GCAAATAGAT CTCCATTACC
4921    CATTGCAAAG GTATCATCAT TTCCATCTAT TAGACCTATA TATCTGACCA GGGTCCTATT
4981    CCAAGACAAC TCTTCTCATC TTCCAGCAGC ATCTTATAGA AAGAAAGATT CTGGGGTCCA
5041    AGAAAGCAGT CATTTCTTAC AAGGAGCCAA AAAAAATAAC CTTTCTTTAG CCATTCTAAC
5101    CTTGGAGATG ACTGGTGATC AAAGAGAGGT TGGCTCCCTG GGGACAAGTG CCACAAATTC
5161    AGTCACATAC AAGAAAGTTG AGAACACTGT TCTCCCGAAA CCAGACTTGC CCAAAACATC

5221    TGGCAAAGTT GAATTGCTTC CAAAAGTTCA CATTTATCAG AAGGACCTAT TCCCTACGGA
5281    AACTAGCAAT GGGTCTCCTG GCCATCTGGA TCTCGTGGAA GGGAGCCTTC TTCAGGGAAC
5341    AGAGGGAGCG ATTAAGTGGA ATGAAGCAAA CAGACCTGGA AAAGTTCCCT TTCTGAGAGT
5401    AGCAACAGAA AGCTCTGCAA AGACTCCCTC CAAGCTATTG GATCCTCTTG CTTGGGATAA
5461    CCACTATGGT ACTCAGATAC CAAAAGAAGA GTGGAAATCC CAAGAGAAGT CACCAGAAAA
5521    AACAGCTTTT AAGAAAAAGG ATACCATTTT GTCCCTGAAC GCTTGTGAAA GCAATCATGC
5581    AATAGCAGCA ATAAATGAGG GACAAAATAA GCCCGAAATA GAAGTCACCT GGGCAAAGCA
5641    AGGTAGGACT GAAAGGCTGT GCTCTCAAAA CCCACCAGTC TTGAAACGCC ATCAACGGGA
5701    AATAACTCGT ACTACTCTTC AGTCAGATCA AGAGGAAATT GACTATGATG ATACCATATC
5761    AGTTGAAATG AAGAAGGAAG ATTTTGACAT TTATGATGAG GATGAAAATC AGAGCCCCCG
5821    CAGCTTTCAA AAGAAAACAC GACACTATTT TATTGCTGCA GTGGAGAGGC TCTGGGATTA
5881    TGGGATGAGT AGCTCCCCAC ATGTTCTAAG AAACAGGGCT CAGAGTGGCA GTGTCCCTCA
5941    GTTCAAGAAA GTTGTTTTCC AGGAATTTAC TGATGGCTCC TTTACTCAGC CCTTATACCG
6001    TGGAGAACTA AATGAACATT TGGGACTCCT GGGGCCATAT ATAAGAGCAG AAGTTGAAGA
6061    TAATATCATG GTAACTTTCA GAAATCAGGC CTCTCGTCCC TATTCCTTCT ATTCTAGCCT
6121    TATTTCTTAT GAGGAAGATC AGAGGCAAGG AGCAGAACCT AGAAAAAACT TTGTCAAGCC
6181    TAATGAAACC AAAACTTACT TTTGGAAAGT GCAACATCAT ATGGCACCCA CTAAAGATGA
6241    GTTTGACTGC AAAGCCTGGG CTTATTTCTC TGATGTTGAC CTGGAAAAAG ATGTGCACTC
6301    AGGCCTGATT GGACCCCTTC TGGTCTGCCA CACTAACACA CTGAACCCTG CTCATGGGAG
6361    ACAAGTGACA GTACAGGAAT TTGCTCTGTT TTTCACCATC TTTGATGAGA CCAAAAGCTG
6421    GTACTTCACT GAAAATATGG AAAGAAACTG CAGGGCTCCC TGCAATATCC AGATGGAAGA
6481    TCCCACTTTT AAAGAGAATT ATCGCTTCCA TGCAATCAAT GGCTACATAA TGGATACACT
6541    ACCTGGCTTA GTAATGGCTC AGGATCAAAG GATTCGATGG TATCTGCTCA GCATGGGCAG
6601    CAATGAAAAC ATCCATTCTA TTCATTTCAG TGGACATGTG TTCACTGTAC GAAAAAAAGA
6661    GGAGTATAAA ATGGCACTGT ACAATCTCTA TCCAGGTGTT TTTGAGACAG TGGAAATGTT
6721    ACCATCCAAA GCTGGAATTT GGCGGGTGGA ATGCCTTATT GGCGAGCATC TACATGCTGG
6781    GATGAGCACA CTTTTTCTGG TGTACAGCAA TAAGTGTCAG ACTCCCCTGG GAATGGCTTC
6841    TGGACACATT AGAGATTTTC AGATTACAGC TTCAGGACAA TATGGACAGT GGGCCCCAAA
6901    GCTGGCCAGA CTTCATTATT CCGGATCAAT CAATGCCTGG AGCACCAAGG AGCCCTTTTC
6961    TTGGATCAAG GTGGATCTGT TGGCACCAAT GATTATTCAC GGCATCAAGA CCCAGGGTGC
7021    CCGTCAGAAG TTCTCCAGCC TCTACATCTC TCAGTTTATC ATCATGTATA GTCTTGATGG
7081    GAAGAAGTGG CAGACTTATC GAGGAAATTC CACTGGAACC TTAATGGTCT TCTTTGGCAA
7141    TGTGGATTCA TCTGGGATAA AACACAATAT TTTTAACCCT CCAATTATTG CTCGATACAT
7201    CCGTTTGCAC CCAACTCATT ATAGCATTCG CAGCACTCTT CGCATGGAGT TGATGGGCTG
7261    TGATTTAAAT AGTTGCAGCA TGCCATTGGG AATGGAGAGT AAAGCAATAT CAGATGCACA
7321    GATTACTGCT TCATCCTACT TTACCAATAT GTTTGCCACC TGGTCTCCTT CAAAAGCTCG
7381    ACTTCACCTC CAAGGGAGGA GTAATGCCTG GAGACCTCAG GTGAATAATC CAAAAGAGTG
7441    GCTGCAAGTG GACTTCCAGA AGACAATGAA AGTCACAGGA GTAACTACTC AGGGAGTAAA
7501    ATCTCTGCTT ACCAGCATGT ATGTGAAGGA GTTCCTCATC TCCAGCAGTC AAGATGGCCA
7561    TCAGTGGACT CTCTTTTTTC AGAATGGCAA AGTAAAGGTT TTTCAGGGAA ATCAAGACTC
7621    CTTCACACCT GTGGTGAACT CTCTAGACCC ACCGTTACTG ACTCGCTACC TTCGAATTCA
7681    CCCCCAGAGT TGGGTGCACC AGATTGCCCT GAGGATGGAG GTTCTGGGCT GCGAGGCACA
7741    GGACCTCTAC
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*The underlined nucleic acids encode a signal peptide.

[0123] FVIII polypeptides include full-length FVIII, full-length FVIII minus Met at the N-terminus, mature FVIII (minus the signal sequence), mature FVIII with an additional Met at the N-terminus, and/or FVIII with a full or partial deletion of the B domain. In certain embodiments, FVIII variants include B domain deletions, whether partial or full deletions.

[0124] The sequence of native mature human FVIII is presented as SEQ ID NO: 65. A native FVIII protein has the following formula: A1-a1-A2-a2-B-a3-A3-C1-C2, where A1, A2, and A3 are the structurally-related "A domains," B is the "B domain," C1 and C2 are the structurally-related "C domains," and a1, a2 and a3 are acidic spacer regions. Referring to the primary amino acid sequence position in SEQ ID NO:65, the A1 domain of human FVIII extends from Ala1 to about Arg336, the a1 spacer region extends from about Met337 to about Val374, the A2 domain extends from about Ala375 to about Tyr719, the a2 spacer region extends from about Glu720 to about Arg740, the B domain extends from about Ser741 to about Arg 1648, the a3 spacer region extends from about Glu1649 to about Arg1689, the A3 domain extends from about Ser1690 to about Leu2025, the C1 domain extends from about Gly2026 to about Asn2072, and the C2 domain extends from about Ser2073 to Tyr2332. Other than specific proteolytic cleavage sites, designation of the locations of the boundaries between the domains and regions of FVIII can vary in different literature references. The boundaries noted herein are therefore designated as approximate by use of the term "about."

[0125] The human FVIII gene was isolated and expressed in mammalian cells (Toole, J. J., et al., Nature 312:342-347 (1984); Gitschier, J., et al., Nature 312:326-330 (1984); Wood, W. I., et al., Nature 312:330-337 (1984); Vehar, G. A., et al., Nature 312:337-342 (1984); WO 87/04187; WO 88/08035; WO 88/03558; and U.S. Pat. No. 4,757,006). The FVIII amino acid sequence was deduced from cDNA as shown in U.S. Pat. No. 4,965,199. In addition, partially or fully B-domain deleted FVIII is shown in U.S. Pat. Nos. 4,994,371 and 4,868,112. In some embodiments, the human FVIII B-domain is replaced with the human Factor V B-domain as shown in U.S. Pat. No. 5,004,803. The cDNA sequence encoding human Factor VIII and amino acid sequence are shown in SEQ ID NOs: 1 and 2, respectively, of US Application Publ. No. 2005/0100990.

[0126] The porcine FVIII sequence is published in Toole, J. J., et al., Proc. Natl. Acad. Sci. USA 83:5939-5942 (1986). Further, the complete porcine cDNA sequence obtained from PCR amplification of FVIII sequences from a pig spleen cDNA library has been reported in Healey, J. F., et al., Blood 88:4209-4214 (1996). Hybrid human/porcine FVIII having substitutions of all domains, all subunits, and specific amino acid sequences were disclosed in U.S. Pat. No. 5,364,771 by Lollar and Runge, and in WO 93/20093. More recently, the nucleotide and corresponding amino acid sequences of the A1 and A2 domains of porcine FVIII and a chimeric FVIII with porcine A1 and/or A2 domains substituted for the corresponding human domains were reported in WO 94/11503. U.S. Pat. No. 5,859,204, Lollar, J. S., also discloses the porcine cDNA and deduced amino acid sequences. U.S. Pat. No. 6,458,563 discloses a B-domain-deleted porcine FVIII.

[0127] U.S. Pat. No. 5,859,204 to Lollar, J. S. reports functional mutants of FVIII having reduced antigenicity and reduced immunoreactivity. U.S. Pat. No. 6,376,463 to Lollar, J. S. also reports mutants of FVIII having reduced immunoreactivity. US Appl. Publ. No. 2005/0100990 to Saenko et al. reports functional mutations in the A2 domain of FVIII.

[0128] In one embodiment, the FVIII (or FVIII portion of a chimeric protein) may be at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a FVIII amino acid sequence of amino acids 1 to 1438 of SEQ ID NO: 67 or amino acids 1 to 2332 of SEQ ID NO: 65 (without a signal sequence) or a FVIII amino acid sequence of amino acids 1 to 19 of SEQ ID NO: 64 and 1 to 1438 of SEQ ID NO: 67 or amino acids 1 to 19 of SEQ ID NO: 64 and amino acids 1 to 2332 of SEQ ID NO: 65 (with a signal sequence), wherein the FVIII has a clotting activity, *e.g.,* activates Factor IX as a cofactor to convert Factor X to activated Factor X. The FVIII (or FVIII portion of a chimeric protein) may be identical to a FVIII amino acid sequence of amino acids 1 to 1438 of

SEQ ID NO: 67 or amino acids 1 to 2332 of SEQ ID NO: 65 (without a signal sequence). The FVIII may further comprise a signal sequence.

[0129] The "B-domain" of FVIII, as used herein, is the same as the B-domain known in the art that is defined by internal amino acid sequence identity and sites of proteolytic cleavage, e.g., residues Ser741-Arg1648 of full-length human FVIII. The other human FVIII domains are defined by the following amino acid residues: A1, residues Ala1-Arg372; A2, residues Ser373-Arg740; A3, residues Ser1690-Asn2019; C1, residues Lys2020-Asn2172; C2, residues Ser2173-Tyr2332. The A3-C1-C2 sequence includes residues Ser1690-Tyr2332. The remaining sequence, residues Glu1649-Arg1689, is usually referred to as the a3 acidic region. The locations of the boundaries for all of the domains, including the B-domains, for porcine, mouse and canine FVIII are also known in the art. In one embodiment, the B domain of FVIII is deleted ("B-domain-deleted factor VIII" or "BDD FVIII"). An example of a BDD FVIII is REFACTO® (recombinant BDD FVIII), which has the same sequence as the Factor VIII portion of the sequence in Table 5. (BDD FVIII heavy chain is double underlined; B domain is italicized; and BDD FVIII light chain is in plain text). A nucleotide sequence encoding Table 6 (SEQ ID NO: 68) is shown in Table 6.

TABLE 5. Amino Acid Sequence of B-domain Deleted Factor VIII (BDD FVIII)

BDD FVIII (SEQ ID NO: 67)

ATRRYYLGAVELSWDYMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTLFVEFTDHLFNIAKPRPPWMGLL
GPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFPGGSHTYVWQVLKEN
GPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKFILLFAVFDEGKSWHSETKNSL
MQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVYWHVIGMGTTPEVHSIFLEGHTFLVRNHRQASLEI
SPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVKVDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRF
DDDNSPSFIQIRSVAKKHPKTWVHYIAAEEEDWDYAPLVLAPDDRSYKSQYLNNGPQRIGRKYKKVRFMAYT
DETFKTREAIQHESGILGPLLYGEVGDTLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDFPIL
PGEIFKYKWTVTVEDGPTKSDPRCLTRYYSSFVNMERDLASGLIGPLLICYKESVDQRGNQIMSDKRNVILF
SVFDENRSWYLTENIQRFLPNPAGVQLEDPEFQASNIMHSINGYVFDSLQLSVCLHEVAYWYILSIGAQTDF
LSVFFSGYTFKHKMVYEDTLTLFPFSGETVFMSMENPGLWILGCHNSDFRNRGMTALLKVSSCDKNTGDYYE
DSYEDISAYLLSKNNAIEPR*SFSQNPPVLKRHQR*EITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQ
SPRSFQKKTRHYFIAAVERLWDYGMSSSPHVLRNRAQSGSVPQFKKVVFQEFTDGSFTQPLYRGELNEHLGL
LGPYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQRQGAEPRKNFVKPNETKTYFWKVQHHMAPTKDEF
DCKAWAYFSDVDLEKDVHSGLIGPLLVCHTNTLNPAHGRQVTVQEFALFFTIFDETKSWYFTENMERNCRAP
CNIQMEDPTFKENYRFHAINGYIMDTLPGLVMAQDQRIRWYLLSMGSNENIHSIHFSGHVFTVRKKEEYKMA
LYNLYPGVFETVEMLPSKAGIWRVECLIGEHLHAGMSTLFLVYSNKCQTPLGMASGHIRDFQITASGQYGQW
APKLARLHYSGSINAWSTKEPFSWIKVDLLAPMIIHGIKTQGARQKFSSLYISQFIIMYSLDGKKWQTYRGN
STGTLMVFFGNVDSSGIKHNIFNPPIIARYIRLHPTHYSIRSTLRMELMGCDLNSCSMPLGMESKAISDAQI
TASSYFTNMFATWSPSKARLHLQGRSNAWRPQVNNPKEWLQVDFQKTMKVTGVTTQGVKSLLTSMYVKEFLI
SSSQDGHQWTLFFQNGKVKVFQGNQDSFTPVVNSLDPPLLTRYLRIHPQSWVHQIALRMEVLGCEAQDLY

TABLE 6. Nucleotide Sequence Encoding BDD FVIII (SEQ ID NO: 68)*

```
 661                                   A TGCAAATAGA GCTCTCCACC TGCTTCTTTC
 721    TGTGCCTTTT GCGATTCTGC TTTAGTGCCA CCAGAAGATA CTACCTGGGT GCAGTGGAAC
 781    TGTCATGGGA CTATATGCAA AGTGATCTCG GTGAGCTGCC TGTGGACGCA AGATTTCCTC
 841    CTAGAGTGCC AAAATCTTTT CCATTCAACA CCTCAGTCGT GTACAAAAAG ACTCTGTTTG
 901    TAGAATTCAC GGATCACCTT TTCAACATCG CTAAGCCAAG GCCACCCTGG ATGGGTCTGC
 961    TAGGTCCTAC CATCCAGGCT GAGGTTTATG ATACAGTGGT CATTACACTT AAGAACATGG
1021    CTTCCCATCC TGTCAGTCTT CATGCTGTTG GTGTATCCTA CTGGAAAGCT TCTGAGGGAG
1081    CTGAATATGA TGATCAGACC AGTCAAAGGG AGAAAGAAGA TGATAAAGTC TTCCCTGGTG
1141    GAAGCCATAC ATATGTCTGG CAGGTCCTGA AAGAGAATGG TCCAATGGCC TCTGACCCAC
1201    TGTGCCTTAC CTACTCATAT CTTTCTCATG TGGACCTGGT AAAAGACTTG AATTCAGGCC
1261    TCATTGGAGC CCTACTAGTA TGTAGAGAAG GGAGTCTGGC CAAGGAAAAG ACACAGACCT
1321    TGCACAAATT TATACTACTT TTTGCTGTAT TTGATGAAGG GAAAAGTTGG CACTCAGAAA
1381    CAAAGAACTC CTTGATGCAG GATAGGGATG CTGCATCTGC TCGGGCCTGG CCTAAAATGC
1441    ACACAGTCAA TGGTTATGTA AACAGGTCTC TGCCAGGTCT GATTGGATGC CACAGGAAAT
1501    CAGTCTATTG GCATGTGATT GGAATGGGCA CCACTCCTGA AGTGCACTCA ATATTCCTCG
1561    AAGGTCACAC ATTTCTTGTG AGGAACCATC GCCAGGCGTC CTTGGAAATC TCGCCAATAA
1621    CTTTCCTTAC TGCTCAAACA CTCTTGATGG ACCTTGGACA GTTTCTACTG TTTTGTCATA
1681    TCTCTTCCCA CCAACATGAT GGCATGGAAG CTTATGTCAA AGTAGACAGC TGTCCAGAGG
1741    AACCCCAACT ACGAATGAAA AATAATGAAG AAGCGGAAGA CTATGATGAT GATCTTACTG
1801    ATTCTGAAAT GGATGTGGTC AGGTTTGATG ATGACAACTC TCCTTCCTTT ATCCAAATTC
1861    GCTCAGTTGC CAAGAAGCAT CCTAAAACTT GGGTACATTA CATTGCTGCT GAAGAGGAGG
1921    ACTGGGACTA TGCTCCCTTA GTCCTCGCCC CCGATGACAG AAGTTATAAA AGTCAATATT
1981    TGAACAATGG CCCTCAGCGG ATTGGTAGGA AGTACAAAAA AGTCCGATTT ATGGCATACA
2041    CAGATGAAAC CTTTAAGACT CGTGAAGCTA TTCAGCATGA ATCAGGAATC TTGGGACCTT
2101    TACTTTATGG GGAAGTTGGA GACACACTGT TGATTATATT TAAGAATCAA GCAAGCAGAC
2161    CATATAACAT CTACCCTCAC GGAATCACTG ATGTCCGTCC TTTGTATTCA AGGAGATTAC
2221    CAAAAGGTGT AAAACATTTG AAGGATTTTC CAATTCTGCC AGGAGAAATA TTCAAATATA
2281    AATGGACAGT GACTGTAGAA GATGGGCCAA CTAAATCAGA TCCTCGGTGC CTGACCCGCT
2341    ATTACTCTAG TTTCGTTAAT ATGGAGAGAG ATCTAGCTTC AGGACTCATT GGCCCTCTCC
2401    TCATCTGCTA CAAAGAATCT GTAGATCAAA GAGGAAACCA GATAATGTCA GACAAGAGGA
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2461    ATGTCATCCT GTTTTCTGTA TTTGATGAGA ACCGAAGCTG GTACCTCACA GAGAATATAC
2521    AACGCTTTCT CCCCAATCCA GCTGGAGTGC AGCTTGAGGA TCCAGAGTTC CAAGCCTCCA
2581    ACATCATGCA CAGCATCAAT GGCTATGTTT TTGATAGTTT GCAGTTGTCA GTTTGTTTGC
2641    ATGAGGTGGC ATACTGGTAC ATTCTAAGCA TTGGAGCACA GACTGACTTC CTTTCTGTCT
2701    TCTTCTCTGG ATATACCTTC AAACACAAAA TGGTCTATGA AGACACACTC ACCCTATTCC
2761    CATTCTCAGG AGAAACTGTC TTCATGTCGA TGGAAAACCC AGGTCTATGG ATTCTGGGGT
2821    GCCACAACTC AGACTTTCGG AACAGAGGCA TGACCGCCTT ACTGAAGGTT TCTAGTTGTG
2881    ACAAGAACAC TGGTGATTAT TACGAGGACA GTTATGAAGA TATTTCAGCA TACTTGCTGA
2941    GTAAAAACAA TGCCATTGAA CCAAGAAGCT TCTCTCAAAA CCCACCAGTC TTGAAACGCC
3001    ATCAACGGGA AATAACTCGT ACTACTCTTC AGTCAGATCA AGAGGAAATT GACTATGATG
3061    ATACCATATC AGTTGAAATG AAGAAGGAAG ATTTTGACAT TTATGATGAG GATGAAAATC
3121    AGAGCCCCCG CAGCTTTCAA AAGAAAACAC GACACTATTT TATTGCTGCA GTGGAGAGGC
3181    TCTGGGATTA TGGGATGAGT AGCTCCCCAC ATGTTCTAAG AAACAGGGCT CAGAGTGGCA
3241    GTGTCCCTCA GTTCAAGAAA GTTGTTTTCC AGGAATTTAC TGATGGCTCC TTTACTCAGC
3301    CCTTATACCG TGGAGAACTA AATGAACATT TGGGACTCCT GGGGCCATAT ATAAGAGCAG
3361    AAGTTGAAGA TAATATCATG GTAACTTTCA GAAATCAGGC CTCTCGTCCC TATTCCTTCT
3421    ATTCTAGCCT TATTTCTTAT GAGGAAGATC AGAGGCAAGG AGCAGAACCT AGAAAAAACT
3481    TTGTCAAGCC TAATGAAACC AAAACTTACT TTTGGAAAGT GCAACATCAT ATGGCACCCA
3541    CTAAAGATGA GTTTGACTGC AAAGCCTGGG CTTATTTCTC TGATGTTGAC CTGGAAAAAG
3601    ATGTGCACTC AGCCCTCATT CGACCCCTTC TGCTCTCCCA CACTAACACA CTGAACCCTC
3661    CTCATGGGAG ACAAGTGACA GTACAGGAAT TTGCTCTGTT TTTCACCATC TTTGATGAGA
3721    CCAAAAGCTG GTACTTCACT GAAAATATGG AAAGAAACTG CAGGGCTCCC TGCAATATCC
3781    AGATGGAAGA TCCCACTTTT AAAGAGAATT ATCGCTTCCA TGCAATCAAT GGCTACATAA
3841    TGGATACACT ACCTGGCTTA GTAATGGCTC AGGATCAAAG GATTCGATGG TATCTGCTCA
3901    GCATGGGCAG CAATGAAAAC ATCCATTCTA TTCATTTCAG TGGACATGTG TTCACTGTAC
3961    GAAAAAAAGA GGAGTATAAA ATGGCACTGT ACAATCTCTA TCCAGGTGTT TTTGAGACAG

4021    TGGAAATGTT ACCATCCAAA GCTGGAATTT GGCGGGTGGA ATGCCTTATT GGCGAGCATC
4081    TACATGCTGG GATGAGCACA CTTTTTCTGG TGTACAGCAA TAAGTGTCAG ACTCCCCTGG
4141    GAATGGCTTC TGGACACATT AGAGATTTTC AGATTACAGC TTCAGGACAA TATGGACAGT
4201    GGGCCCCAAA GCTGGCCAGA CTTCATTATT CCGGATCAAT CAATGCCTGG AGCACCAAGG
4261    AGCCCTTTTC TTGGATCAAG GTGGATCTGT TGGCACCAAT GATTATTCAC GGCATCAAGA
4321    CCCAGGGTGC CCGTCAGAAG TTCTCCAGCC TCTACATCTC TCAGTTTATC ATCATGTATA
4381    GTCTTGATGG GAAGAAGTGG CAGACTTATC GAGGAAATTC CACTGGAACC TTAATGGTCT
4441    TCTTTGGCAA TGTGGATTCA TCTGGGATAA AACACAATAT TTTTAACCCT CCAATTATTG
4501    CTCGATACAT CCGTTTGCAC CCAACTCATT ATAGCATTCG CAGCACTCTT CGCATGGAGT
4561    TGATGGGCTG TGATTTAAAT AGTTGCAGCA TGCCATTGGG AATGGAGAGT AAAGCAATAT
4621    CAGATGCACA GATTACTGCT TCATCCTACT TTACCAATAT GTTTGCCACC TGGTCTCCTT
4681    CAAAAGCTCG ACTTCACCTC CAAGGGAGGA GTAATGCCTG GAGACCTCAG GTGAATAATC
4741    CAAAAGAGTG GCTGCAAGTG GACTTCCAGA AGACAATGAA AGTCACAGGA GTAACTACTC
4801    AGGGAGTAAA ATCTCTGCTT ACCAGCATGT ATGTGAAGGA GTTCCTCATC TCCAGCAGTC
4861    AAGATGGCCA TCAGTGGACT CTCTTTTTTC AGAATGGCAA AGTAAAGGTT TTTCAGGGAA
4921    ATCAAGACTC CTTCACACCT GTGGTGAACT CTCTAGACCC ACCGTTACTG ACTCGCTACC
4981    TTCGAATTCA CCCCCAGAGT TGGGTGCACC AGATTGCCCT GAGGATGGAG GTTCTGGGCT
5041    GCGAGGCACA GGACCTCTAC
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*The underlined nucleic acids encode a signal peptide.

[0130] A "B-domain-deleted FVIII" may have the full or partial deletions disclosed in U.S. Pat. Nos. 6,316,226, 6,346,513, 7,041,635, 5,789,203, 6,060,447, 5,595,886, 6,228,620, 5,972,885, 6,048,720, 5,543,502, 5,610,278, 5,171,844, 5,112,950, 4,868,112, and 6,458,563. In some embodiments, a B-domain-deleted FVIII sequence of the present invention comprises any one of the deletions disclosed at col. 4, line 4 to col. 5, line 28 and Examples 1-5 of U.S. Pat. No. 6,316,226 (also in US 6,346,513). In another embodiment, a B-domain deleted Factor VIII is the S743/Q1638 B-domain deleted Factor VIII (SQ BDD FVIII) *(e.g.,* Factor VIII having a deletion from amino acid 744 to amino acid 1637, *e.g.,* Factor VIII having amino acids 1-743 and amino acids 1638-2332 of SEQ ID NO: 65, *i.e.,* SEQ ID NO: 67). In some embodiments, a B-domain-deleted FVIII of the present invention has a deletion disclosed at col. 2, lines 26-51 and examples 5-8 of U.S. Patent No. 5,789,203 (also US 6,060,447, US 5,595,886, and US 6,228,620). In some embodiments, a B-domain-deleted Factor VIII has a deletion described in col. 1, lines 25 to col. 2, line 40 of US Patent No. 5,972,885; col. 6, lines 1-22 and example 1 of U.S. Patent no. 6,048,720; col. 2, lines 17-46 of U.S. Patent No. 5,543,502; col. 4, line 22 to col. 5, line 36 of U.S. Patent no. 5,171,844; col. 2, lines 55-68, figure 2, and example 1 of U.S. Patent No. 5,112,950; col. 2, line 2 to col. 19, line 21 and table 2 of U.S. Patent No. 4,868,112; col. 2, line 1 to col. 3, line 19, col. 3, line 40 to col. 4, line 67, col. 7, line 43 to col. 8, line 26, and col. 11, line 5 to col. 13, line 39 of U.S. Patent no. 7,041,635; or col. 4, lines 25-53, of U.S. Patent No. 6,458,563. In some embodiments, a B-domain-deleted FVIII has a deletion of most of the B domain, but still contains amino-terminal sequences of the B domain that are essential for *in vivo* proteolytic processing of the primary translation product into two polypeptide chain, as disclosed in WO 91/09122. In some embodiments, a B-domain-deleted FVIII is constructed with a deletion of amino acids 747-1638, *i.e.,* virtually a complete deletion of the B domain. Hoeben R.C., et al. J. Biol. Chem. 265

(13): 7318-7323 (1990). A B-domain-deleted Factor VIII may also contain a deletion of amino acids 771-1666 or amino acids 868-1562 of FVIII. Meulien P., et al. Protein Eng. 2(4): 301-6 (1988). Additional B domain deletions that are part of the invention include: deletion of amino acids 982 through 1562 or 760 through 1639 (Toole et al., Proc. Natl. Acad. Sci. U.S.A. (1986) 83, 5939-5942)), 797 through 1562 (Eaton, et al. Biochemistry (1986) 25:8343-8347)), 741 through 1646 (Kaufman (PCT published application No. WO 87/04187)), 747-1560 (Sarver, et al., DNA (1987) 6:553-564)), 741 through 1648 (Pasek (PCT application No.88/00831)), or 816 through 1598 or 741 through 1648 (Lagner (Behring Inst. Mitt. (1988) No 82:16-25, EP 295597)). In other embodiments, BDD FVIII includes a FVIII polypeptide containing fragments of the B-domain that retain one or more N-linked glycosylation sites, e.g., residues 757, 784, 828, 900, 963, or optionally 943, which correspond to the amino acid sequence of the full-length FVIII sequence. Examples of the B-domain fragments include 226 amino acids or 163 amino acids of the B-domain as disclosed in Miao, H.Z., et al., Blood 103(a): 3412-3419 (2004), Kasuda, A, et al., J. Thromb. Haemost. 6: 1352-1359 (2008), and Pipe, S.W., et al., J. Thromb. Haemost. 9: 2235-2242 (2011) *(i.e.,* the first 226 amino acids or 163 amino acids of the B domain are retained). In still other embodiments, BDD FVIII further comprises a point mutation at residue 309 (from Phe to Ser) to improve expression of the BDD FVIII protein. *See* Miao, H.Z., et al., Blood 103(a): 3412-3419 (2004). In still other embodiments, the BDD FVIII includes a FVIII polypeptide containing a portion of the B-domain, but not containing one or more furin cleavage sites *(e.g.,* Arg1313 and Arg 1648). *See* Pipe, S.W., et al., J. Thromb. Haemost. 9: 2235-2242 (2011). Each of the foregoing deletions may be made in any FVIII sequence.

[0131] In some embodiments, the FVIII has a partial B-domain. In some embodiments, the FVIII protein with a partial B-domain is FVIII198. FVIII198 is a partial B-domain containing single chain FVIIIFc molecule-226N6. Number 226 represents the N-terminus 226 amino acid of the FVIII B-domain, and N6 represents six N-glycosylation sites in the B-domain.

[0132] In one embodiment, FVIII is cleaved right after Arginine at amino acid 1648 (in full-length Factor VIII or SEQ ID NO: 65), amino acid 754 (in the S743/Q1638 B-domain deleted Factor VIII or SEQ ID NO: 67), or the corresponding Arginine residue (in other variants), thereby resulting in a heavy chain and a light chain. In another embodiment, FVIII comprises a heavy chain and a light chain, which are linked or associated by a metal ion-mediated non-covalent bond.

[0133] In other embodiments, FVIII is a single chain FVIII that has not been cleaved right after Arginine at amino acid 1648 (in full-length FVIII or SEQ ID NO: 65), amino acid 754 (in the S743/Q1638 B-domain-deleted FVIII or SEQ ID NO: 67), or the corresponding Arginine residue (in other variants). A single chain FVIII may comprise one or more amino acid substitutions. In one embodiment, the amino acid substitution is at a residue corresponding to residue 1648, residue 1645, or both of full-length mature Factor VIII polypeptide (SEQ ID NO: 65) or residue 754, residue 751, or both of SQ BDD Factor VIII (SEQ ID NO: 67). The amino acid substitution can be any amino acids other than Arginine, e.g., isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, selenocysteine, serine, tyrosine, histidine, ornithine, pyrrolysine, or taurine.

[0134] FVIII can further be cleaved by thrombin and then activated as FVIIIa, serving as a cofactor for activated Factor IX (FIXa). And the activated FIX together with activated FVIII forms a Xase complex and converts Factor X to activated Factor X (FXa). For activation, FVIII is cleaved by thrombin after three Arginine residues, at amino acids 372, 740, and 1689 (corresponding to amino acids 372, 740, and 795 in the B-domain deleted FVIII sequence), the cleavage generating FVIIIa having the 50kDa A1, 43kDa A2, and 73kDa A3-C1-C2 chains. In one embodiment, the FVIII protein useful for the present invention is non-active FVIII. In another embodiment, the FVIII protein is an activated FVIII.

[0135] The protein having FVIII polypeptide linked to or associated with the VWF protein can comprise a sequence at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 65 or 67, wherein the sequence has the FVIII clotting activity, e.g., activating Factor IX as a cofactor to convert Factor X to activated Factor X (FXa).

[0136] "Hybrid" or "chimeric" polypeptides and proteins, as used herein, includes a combination of a first polypeptide chain, e.g., the VWF protein fused to an XTEN sequence having less than 288 amino acids and a first Ig constant region or a portion thereof, with a second polypeptide chain, *e.g.,* a FVIII protein fused to a second Ig constant region or a portion thereof, thereby forming a heterodimer. The first polypeptide and the second polypeptide in a hybrid may be associated with each other via protein-protein interactions, such as charge-charge or hydrophobic interactions. A first polypeptide may comprise a VWF protein-XTEN-Fc fusion protein, and a second polypeptide may comprise a FVIII-Fc fusion protein, making the hybrid a heterodimer, wherein the XTEN contains less than 288 amino acids. The first polypeptide may comprise a VWF protein-XTEN-Fc fusion protein, and the second polypeptide may comprise a FVIII(X)-Fc fusion protein, making the hybrid a heterodimer, wherein the XTEN contains less than 288 amino acids. The first polypeptide and the second polypeptide can be associated through a covalent bond, *e.g.,* a disulfide bond, between the first Fc region and the second Fc region. The first polypeptide and the second polypeptide can further be associated with each other by binding between the VWF fragment and the FVIII protein.

[0137] A FVIII protein useful in the present invention can include FVIII having one or more additional XTEN sequences, which do not affect the FVIII coagulation activity. Such XTEN sequences can be fused to the C-terminus or N-terminus of the FVIII protein or inserted between one or more of the two amino acid residues in the FVIII protein while the insertions do not affect the FVIII coagulation activity or FVIII function. In one embodiment, the insertions improve pharmacokinetic properties of the FVIII protein (e.g., half-life). In another embodiment, the insertions can be multiple insertions, e.g., more than two, three, four, five, six, seven, eight, nine, or ten insertions. Examples of the insertion sites include, but are not limited to, the sites listed in Tables 7, 8, 9, 10, 11, 12, 13, 14, 15 or any combinations thereof.

[0138] The FVIII protein linked to one or more XTEN sequences can be represented as FVIII(X2) or $FVIII_{(a\rightarrow b)}$-X-$FVIII_{(c\rightarrow d)}$, wherein $FVIII_{(a\rightarrow b)}$ comprises, consists essentially of, or consists of a first portion of a FVIII protein from amino acid residue "a" to amino acid residue "b"; X2 comprises, consists essentially of, or consists of one or more XTEN sequences, $FVIII_{(c\rightarrow d)}$ comprises, consists essentially of, or consists of a second portion of a FVIII protein from amino acid residue "c" to amino acid residue "d";

1. a is the N-terminal amino acid residue of the first portion of the FVIII protein,
2. b is the C-terminal amino acid residue of the first portion of the FVIII protein but is also the N-terminal amino acid residue of the two amino acids of an insertion site in which the XTEN sequence is inserted,
3. c is the N-terminal amino acid residue of the second portion of the FVIII protein but is also the C-terminal amino acid residue of the two amino acids of an insertion site in which the XTEN sequence is inserted, and
4. d is the C-terminal amino acid residue of the FVIII protein, and

wherein the first portion of the FVIII protein and the second portion of the FVIII protein are not identical to each other and are of sufficient length together such that the FVIII protein has a FVIII coagulation activity.

[0139] In one embodiment, the first portion of the FVIII protein and the second portion of the FVIII protein are fragments of SEQ ID NO: 65 [full length mature FVIII sequence] or SEQ ID NO: 67 [B-domain deleted FVIII], e.g., N-terminal portion and C-terminal portion, respectively. In certain embodiments, the first portion of the FVIII protein comprises the A1 domain and the A2 domain of the FVIII protein. The second portion of the FVIII protein comprises the A3 domain, the C1 domain, and optionally the C2 domain. In yet other embodiments, the first portion of the FVIII protein comprises the A1 domain and A2 domain, and the second portion of the FVIII protein comprises a portion of the B domain, the A3 domain, the C1 domain, and optionally the C2 domain. In still other embodiments, the first portion of the FVIII protein comprises the A1 domain, A2 domain, and a portion of the B domain of the FVIII protein, and the second portion of the FVIII protein comprises the A3 domain, the C1 domain, and optionally the C2 domain. In still other embodiments, the first portion of the FVIII protein comprises the A1 domain, A2 domain, and a first portion of the B domain of the FVIII protein. The second portion of the FVIII protein comprises a second portion of the B domain, the A3 domain, the C1 domain, and optionally the C2 domain. In some embodiments, the two amino acids ("b" and "c") can be any one or more of the amino acid

residues insertion sites shown in Tables 7, 8, 9, 10, 11, 12, 13, 14, and 15. For example, "b" can be the amino acid residue immediately upstream of the site in which one or more XTEN sequences are inserted or linked, and "c" can be the amino acid residue immediately downstream of the site in which the one or more XTEN sequences are inserted or linked. In some embodiments, "a" is the first mature amino acid sequence of a FVIII protein, and "d" is the last amino acid sequence of a FVIII protein. For example, $FVIII_{(a\rightarrow b)}$ can be an amino acid sequence at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 1 to 745 of SEQ ID NO: 67 [B domain deleted FVIII amino acid sequence] or SEQ ID NO: 65 [full length FVIII] and $FVIII_{(c\rightarrow d)}$ can be amino acids 746 to 1438 of SEQ ID NO: 67 or amino acids 1641 to 2332 of SEQ ID NO: 65, respectively.

[0140] In some aspects, the insertion site in the FVIII protein is located in one or more domains of the FVIII protein, which is the N-terminus, the A1 domain, the A2 domain, the A3 domain, the B domain, the C1 domain, the C2 domain, the C-terminus, or two or more combinations thereof or between two domains of the FVIII protein, which are the A1 domain and a1 acidic region, and the a1 acidic region and A2 domain, the A2 domain and a2 acidic region, the a2 acidic region and B domain, the B domain and A3 domain, and the A3 domain and C1 domain, the C1 domain and C2 domain, or any combinations thereof. For example, the insertion sites in which the XTEN sequence can be inserted are selected from the group consisting of the N-terminus and A1 domain, the N-terminus and A2 domain, the N-terminus and A3 domain, the N-terminus and B domain, the N-terminus and C1 domain, the N-terminus and C2 domain, the N-terminus and the C-terminus, the A1 and A2 domains, the A1 and A3 domains, the A1 and B domains, the A1 and C1 domains, the A1 and C2 domains, the A1 domain and the C-terminus, the A2 and A3 domains, the A2 and B domains, the A2 and C1 domains, the A2 and C2 domains, the A2 domain and the C-terminus, the A3 and B domains, the A3 and C1 domains, the A3 and C2 domains, the A3 domain and the C-terminus, the B and C1 domains, the B and C2 domains, the B domain and the C-terminus, the C1 and C2 domains, the C1 and the C-terminus, the C2 domain, and the C-terminus, and two or more combinations thereof. Non-limiting examples of the insertion sites are listed in Tables 7, 8, 9, 10, 11, 12, 13, 14, and 15.

[0141] The FVIII protein, in which the XTEN sequence is inserted immediately downstream of one or more amino acids *(e.g.,* one or more XTEN insertion sites) in the FVIII protein or linked at the C-terminus or the N-terminus, retains the FVIII activity after linkage to or insertion by the XTEN sequence. The XTEN sequence can be inserted in the FVIII protein once or more than once, twice, three times, four times, five times, or six times such that the insertions do not affect the FVIII activity (*i.e.*, the FVIII protein still retains the coagulation property).

[0142] The FVIII protein useful in the present invention can be linked to one or more XTEN polypeptides at the N-terminus or C-terminus of the FVIII protein by an optional linker or inserted immediately downstream of one or more amino acids *(e.g.,* one or more XTEN insertion sites) in the FVIII protein by one or more optional linkers. In one embodiment, the two amino acid residues in which the XTEN sequence is inserted or the amino acid residue to which the XTEN sequence is linked correspond to the two or one amino acid residues of SEQ ID NO: 65 [full length mature FVIII] selected from the group consisting of the residues in Table 7, Table 8, Table 9, and Table 10 and any combinations thereof.

[0143] In other embodiments, at least one XTEN sequence is inserted in any one or more XTEN insertion sites disclosed herein or any combinations thereof. In one aspect, at least one XTEN sequence is inserted in one or more XTEN insertion sites disclosed in one or more amino acids disclosed in Table 7.

TABLE 7: Exemplary XTEN Insertion Sites

No.	XTEN Insertion Point*	Insertion Residue	FVIII BDD Downstream Sequence	FVIII Domain
1	0	(N-terminus)	ATR	A1
2	3	R	RYY	A1
3	17	M	QSD	A1
4	18	Q	SDL	A1
5	22	G	ELP	A1

No.	XTEN Insertion Point*	Insertion Residue	FVIII BDD Downstream Sequence	FVIII Domain
6	24	L	PVD	A1
7	26	V	DAR	A1
8	28	A	RFP	A1
9	32	P	RVP	A1
10	38	F	PFN	A1
11	40	F	NTS	A1
12	41	N	TSV	A1
13	60	N	IAK	A1
14	61	I	AKP	A1
15	65	R	PPW	A1
16	81	Y	DTV	A1
17	111	G	AEY	A1
18	116	D	QTS	A1
19	119	s	QRE	A1
20	120	Q	REK	A1
21	128	V	FPG	A1
22	129	F	PGG	A1
23	130	P	GGS	A1
24	182	G	SLA	A1
25	185	A	KEK	A1
26	188	K	TQT	A1
27	205	G	KSW	A1
28	210	s	ETK	A1
29	211	E	TKN	A1
30	216	L	MQD	A1
31	220	R	DAA	A1
32	222	A	ASA	A1
33	223	A	SAR	A1
34	224	s	ARA	A1
35	230	K	MHT	A1
36	243	P	GLI	A1
37	244	G	LIG	A1
38	250	R	KSV	A1
39	318	D	GME	A1
40	333	P	QLR	A1
42	334	Q	LRM	A1
43	336	R	MKN	a1
44	339	N	NEE	a1
45	345	D	YDD	a1
46	357	V	VRF	a1

No.	XTEN Insertion Point*	Insertion Residue	FVIII BDD Downstream Sequence	FVIII Domain
47	367	S	FIQ	a1
48	370	S	RPY	a1
49	375	A	KKH	A2
50	376	K	KHP	A2
51	378	H	PKT	A2
52	399	V	LAP	A2
53	403	D	DRS	A2
54	405	R	SYK	A2
55	409	s	QYL	A2
56	416	P	QRI	A2
57	434	E	TFK	A2
58	438	T	REA	A2
59	441	A	IQH	A2
60	442	I	QHE	A2
61	463	I	IFK	A2
62	487	Y	SRR	A2
63	490	R	LPK	A2
64	492	P	KGV	A2
65	493	K	GVK	A2
66	494	G	VKH	A2
67	500	D	FPI	A2
68	506	G	EIF	A2
69	518	E	DGP	A2
70	556	K	ESV	A2
71	565	Q	IMS	A2
72	566	I	MSD	A2
73	598	P	AGV	A2
74	599	A	GVQ	A2
75	603	L	EDP	A2
76	616	s	ING	A2
77	686	G	LWI	A2
78	713	K	NTG	A2
79	719	Y	EDS	A2
80	730	L	LSK	A2
81	733	K	NNA	A2
82	745	N	PPV**	B
83	1640	P	PVL	B
84	1652	R	TTL	B
85	1656	Q	SDQ	A3
86	1685	N	QSP	A3

No.	XTEN Insertion Point*	Insertion Residue	FVIII BDD Downstream Sequence	FVIII Domain
87	1711	M	SSS	A3
88	1713	s	SPH	A3
89	1720	N	RAQ	A3
90	1724	s	GSV	A3
91	1725	G	SVP	A3
92	1726	s	VPQ	A3
93	1741	G	SFT	A3
94	1744	T	QPL	A3
95	1749	R	GEL	A3
96	1773	V	TFR	A3
97	1792	Y	EED	A3
98	1793	E	EDQ	A3
99	1796	Q	RQG	A3
100	1798	Q	GAE	A3
101	1799	G	AEP	A3
102	1802	P	RKN	A3
103	1803	R	KNF	A3
104	1807	V	KPN	A3
105	1808	K	PNE	A3
106	1827	K	DEF	A3
107	1844	E	KDV	A3
108	1861	N	TLN	A3
109	1863	L	NPA	A3
110	1896	E	RNC	A3
111	1900	R	APC	A3
112	1904	N	IQM	A3
113	1905	I	QME	A3
114	1910	P	TFK	A3
115	1920	A	ING	A3
116	1937	D	QRI	A3
117	1981	G	VFE	A3
118	2019	N	KCQ	A3
119	2020	K	CQT	C1
120	2044	G	QWA	C1
121	2068	F	SWI	C1
122	2073	V	DLL	C1
123	2090	R	QKF	C1
124	2092	K	FSS	C1
125	2093	F	SSL	C1
126	2111	K	WQT	C1
127	2115	Y	RGN	C1

No.	XTEN Insertion Point*	Insertion Residue	FVIII BDD Downstream Sequence	FVIII Domain
128	2120	T	GTL	C1
129	2125	V	FFG	C1
130	2171	L	NSC	C1
131	2173	s	CSM	C2
132	2188	A	QIT	C2
133	2223	V	NNP	C2
134	2224	N	NPK	C2
135	2227	K	EWL	C2
136	2268	G	HQW	C2
137	2277	N	GKV	C2
138	2278	G	KVK	C2
139	2290	F	TPV	C2
140	2332	Y	C terminus of FVIII	CT

* Indicates an insertion point for XTEN based on the amino acid number of mature full-length human FVIII, wherein the insertion could be either on the N- or C-terminal side of the indicated amino acid.

[0144] In some embodiments, one or more XTEN sequences are inserted within about six amino acids up or down from amino acids 32, 220, 224, 336, 339, 399, 416, 603, 1656, 1711, 1725, 1905, or 1910, corresponding to SEQ ID NO: 65 or any combinations thereof.

TABLE 8. Exemplary XTEN Insertion Ranges

No.	XTEN Insertion Point	Insertion Residue	FVIII BDD Downstream Sequence	FVIII Domain	Distance from insertion residue*
9	32	P	RVP	A1	-3, +6
31	220	R	DAA	A1	-
34	224	s	ARA	A1	+5
43	336	R	MKN	a1	-1, +6
44	339	N	NEE	a1	-4, +5
52	399	V	LAP	A2	-6, +3
56	416	P	QRI	A2	+6
75	603	L	EDP	A2	_6, +6
85	1656	Q	SDQ	B	-3, +6
87	1711	M	SSS	A3	-6, +1
91	1725	G	SVP	A3	+6
113	1905	I	QME	A3	+6
114	1910	P	TFK	A3	-5, +6

*Distance from insertion residue refers to the relative number of amino acids away from the N-terminus (negative numbers) or C-terminus (positive numbers) of the designated insertion residue (residue "0") where an insertion may be made. The designation "-x" refers to an insertion site which is x amino acids away on the N-terminal side of the designated insertion residue. Similarly, the designation "+x" refers to an insertion site which is x amino acids away on the C-terminal side of the designated insertion residue.

For example, "-1, +2" indicates that the insertion is made at the N-terminus or C-terminus of amino acid residues denoted -1, 0, +1 or +2.

[0145] In other embodiments, one or more XTEN sequences are inserted immediately down stream of one or more amino acids corresponding to the full-length mature human FVIII selected from the group consisting of one or more insertion sites in Table 9.

TABLE 9. Exemplary XTEN Insertion Sites or Ranges

No.	XTEN Insertion Point Range*	First Insertion Residue	FVIII Domain
3	18-32	Q	A1
8	40	F	A1
18	211-224	E	A1
27	336-403	R	A1, A2
43	599	A	A2
47	745-1640	N	B
50	1656-1728	Q	B, a3, A3
57	1796-1804	R	A3
65	1900-1912	R	A3
81	2171-2332	L	C1, C2

* indicates range of insertion sites numbered relative to the amino acid number of mature human FVIII

[0146] In yet other embodiments, one or more XTENs are inserted in the B domain of FVIII. In one example, an XTEN is inserted between amino acids 740 and 1640 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 740 and 1640 is optionally not present. In another example, an XTEN is inserted between amino acids 741 and 1690 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 740 and 1690 is optionally not present. In other examples, an XTEN is inserted between amino acids 741 and 1648 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 741 and 1648 is optionally not present. In yet other examples, an XTEN is inserted between amino acids 743 and 1638 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 743 and 1638 is optionally not present. In still other examples, an XTEN is inserted between amino acids 745 and 1656 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 745 and 1656 is optionally not present. In some examples, an XTEN is inserted between amino acids 745 and 1657 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 745 and 1657 is optionally not present. In certain examples, an XTEN is inserted between amino acids 745 and 1667 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 745 and 1667 is optionally not present. In still other examples, an XTEN is inserted between amino acids 745 and 1686 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 745 and 1686 is optionally not present. In some other examples, an XTEN is inserted between amino acids 747 and 1642 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 747 and 1642 is optionally not present. In still other examples, an XTEN is inserted between amino acids 751 and 1667 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 751 and 1667 is optionally not present.

[0147] In some embodiments, one or more XTENs are inserted in one or more amino acids immediately downstream of an amino acid of an insertion site selected from the group consisting of the amino acid residues in Table 10.

TABLE 10: FVIII XTEN insertion sites and construct designations

Construct Number	Domain	Upstream Residue No.*	Downstream Residue No.*	Upstream Sequence	Downstream Sequence
F8X-1	A1	3	4	ATR	RYY
F8X-2	A1	18	19	YMQ	SDL
F8X-3	A1	22	23	DLG	ELP
F8X-4	A1	26	27	LPV	DAR
F8X-5	A1	40	41	FPF	NTS
F8X-6	A1	60	61	LFN	IAK
F8X-7	A1	116	117	YDD	QTS
F8X-8	A1	130	131	VFP	GGS
F8X-9	A1	188	189	KEK	TQT
F8X-10	A1	216	217	NSL	MQD
F8X-11	A1	230	231	WPK	MHT
F8X-12	A1	333	334	EEP	QLR
F8X-13	A2	375	376	SVA	KKH
F8X-14	A2	403	404	APD	DRS
F8X-15	A2	442	443	EAI	QHE
F8X-16	A2	490	491	RRL	PKG
F8X-17	A2	518	519	TVE	DGP
F8X-18	A2	599	600	NPA	GVQ
F8X-19	A2	713	714	CDK	NTG
F8X-20	BD	745	746	SQN	PPV
F8X-21	BD	745	746	SQN	PPV
F8X-22	BD**	745	746	SQN	PPV
F8X-23	A3	1720	1721	APT	KDE
F8X-24	A3	1796	1797	EDQ	RQG
F8X-25	A3	1802	1803	AEP	RKN
F8X-26	A3	1827	1828	PTK	DEF
F8X-27	A3	1861	1862	HTN	TLN
F8X-28	A3	1896	1897	NME	RNC
F8X-29	A3	1900	1901	NCR	APC
F8X-30	A3	1904	1905	PCN	IQM
F8X-31	A3	1937	1938	AQD	QRI
F8X-32	C1	2019	2020	YSN	KCQ
F8X-33	C1	2068	2069	EPF	SWI
F8X-34	C1	2111	2112	GKK	WQT
F8X-35	C1	2120	2121	NST	GTL
F8X-36	C2	2171	2172	CDL	NSC
F8X-37	C2	2188	2189	SDA	QIT
F8X-38	C2	2227	2228	NPK	EWL
F8X-39	C2	2277	2278	FQN	GKV
F8X-40	CT	2332	NA	DLY	NA

Construct Number	Domain	Upstream Residue No.*	Downstream Residue No.*	Upstream Sequence	Downstream Sequence
F8X-41	CT	2332	NA	DLY	NA
F8X-42	A1	3	4	ATR	ATR
pSD0001	A2	403	404		
pSD0002	A2	599	600		
pSD0021	N-term	0	1		
pSD0022	A1	32	33		
pSD0023	A1	65	66		
pSD0024	A1	81	82		
pSD0025	A1	119	120		
pSD0026	A1	211	212		
pSD0027	A1	220	221		
pSD0028	A1	224	225		
pSD0029	A1	336	337		
pSD0030	A1	339	340		
pSD0031	A2	378	379		
pSD0032	A2	399	400		
pSD0033	A2	409	410		
pSD0034	A2	416	417		
pSD0035	A2	487	488		
pSD0036	A2	494	495		
pSD0037	A2	500	501		
pSD0038	A2	603	604		
pSD0039	A3	1656	1657		
pSD0040	A3	1711	1712		
pSD0041	A3	1725	1726		
pSD0042	A3	1749	1750		
pSD0043	A3	1905	1906		
pSD0044	A3	1910	1911		
pDS0062	A3	1900	1901		
* Indicates the amino acid number of the mature FVIII protein					

[0148] In one embodiment, the one or more XTEN insertion sites are located within one or more surface-exposed, flexible loop structure of the FVIII protein *(e.g.,* a permissive loop). For example, at least one XTEN sequence can be inserted in each FVIII "A" domain comprising at least two "permissive loops" into which at least one XTEN polypeptide can be inserted without eliminating procoagulant activity of the recombinant protein, or the ability of the recombinant proteins to be expressed in vivo or *in vitro* in a host cell. The permissive loops are regions that allow insertion of at least one XTEN sequence with, among other attributes, high surface or solvent exposure and high conformational flexibility. The A1 domain comprises a permissive loop-1 (A1-1) region and a permissive loop-2 (A1-2) region, the A2 domain comprises a permissive loop-1 (A2-1) region and a permissive loop-2 (A2-2) region, the A3 domain comprises a permissive loop-1 (A3-1) region and a permissive loop-2 (A3-2) region.

[0149] In one aspect, a first permissive loop in the FVIII A1 domain (A1-1) is located between beta strand 1 and beta strand 2, and a second permissive loop in the FVIII A2 domain (A1-2) is located between beta strand 11 and beta strand 12. A first permissive loop in the FVIII A2 domain (A2-1) is located between beta strand 22 and beta strand 23, and a second permissive loop in the FVIII A2 domain (A2-2) is located between beta strand 32 and beta strand 33. A first permissive loop in the FVIII A3 domain (A3-1) is located between beta strand 38 and beta strand 39, and a second permissive loop in the FVIII A3 (A3-2) is located between beta strand 45 and beta strand 46. In certain aspects, the surface-exposed, flexible loop structure comprising A1-1 corresponds to a region in native mature human FVIII from about amino acid 15 to about amino acid 45 of SEQ ID NO: 65, *e.g.,* from about amino acid 18 to about amino acid 41 of SEQ ID NO: 65. In other aspects, the surface-exposed, flexible loop structure comprising A1-2 corresponds to a region in native mature human FVIII from about amino acid 201 to about amino acid 232 of SEQ ID NO: 65, *e.g.,* from about amino acid 218 to about amino acid 229 of SEQ ID NO: 65. In yet other aspects, the surface-exposed, flexible loop structure comprising A2-1 corresponds to a region in native mature human FVIII from about amino acid 395 to about amino acid 421 of SEQ ID NO: 65, *e.g.* from about amino acid 397 to about amino acid 418 of SEQ ID NO: 65. In still other embodiments, the surface-exposed, flexible loop structure comprising A2-2 corresponds to a region in native mature human FVIII from about amino acid 577 to about amino acid 635 of SEQ ID NO: 65, *e.g.,* from about amino acid 595 to about amino acid 607 of SEQ ID NO: 65. In certain aspects the surface-exposed, flexible loop structure comprising A3-1 corresponds to a region in native mature human FVIII from about amino acid 1705 to about amino acid 1732 of SEQ ID NO: 65, *e.g.,* from about amino acid 1711 to about amino acid 1725 of SEQ ID NO: 65. In yet other aspects, the surface-exposed, flexible loop structure comprising A3-2 corresponds to a region in native mature human FVIII from about amino acid 1884 to about amino acid 1917 of SEQ ID NO: 65, *e.g.,* from about amino acid 1899 to about amino acid 1911 of SEQ ID NO: 65.

[0150] In another embodiment, the one or more amino acids in which at least one XTEN sequence is inserted is located within a3 domain, e.g., amino acids 1649 to 1689, corresponding to full-length mature FVIII polypeptide. In a particular embodiment, an XTEN sequence is inserted between amino acids 1656 and 1657 of SEQ ID NO: 65 (full-length mature FVIII). In a specific embodiment, a FVIII protein comprising an XTEN sequence inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 65 further comprises a deletion from amino acid 745 to amino acid 1656 corresponding to SEQ ID NO: 65.

[0151] In some embodiments, the one or more insertion sites for one or more XTEN insertions are immediately downstream of one or more amino acids corresponding to mature full-length FVIII, selected from the group consisting of:

(1) amino acid 3,	(2) amino acid 18,	(3) amino acid 22,
(4) amino acid 26,	(5) amino acid 32,	(6) amino acid 40,
(7) amino acid 60,	(8) amino acid 65,	(9) amino acid 81,
(10) amino acid 116,	(11) amino acid 119,	(12) amino acid 130,
(13) amino acid 188,	(14) amino acid 211,	(15) amino acid 216,
(16) amino acid 220,	(17) amino acid 224,	(18) amino acid 230,
(19) amino acid 333,	(20) amino acid 336,	(21) amino acid 339,
(22) amino acid 375,	(23) amino acid 399,	(24) amino acid 403,
(25) amino acid 409,	(26) amino acid 416,	(26) amino acid 442,
(28) amino acid 487,	(29) amino acid 490,	(30) amino acid 494,
(31) amino acid 500,	(32) amino acid 518,	(33) amino acid 599,
(34) amino acid 603,	(35) amino acid 713,	(36) amino acid 745,
(37) amino acid 1656,	(38) amino acid 1711,	(39) amino acid 1720,
(40) amino acid 1725,	(41) amino acid 1749,	(42) amino acid 1796,
(43) amino acid 1802,	(44) amino acid 1827,	(45) amino acid 1861,

(46) amino acid 1896,	(47) amino acid 1900,	(48) amino acid 1904,
(49) amino acid 1905,	(50) amino acid 1910,	(51) amino acid 1937,
(52) amino acid 2019,	(53) amino acid 2068,	(54) amino acid 2111,
(55) amino acid 2120,	(56) amino acid 2171,	(57) amino acid 2188,
(58) amino acid 2227,	(59) amino acid 2277, and	
(60) two or more combinations thereof.		

[0152] In one embodiment, a FVIII protein useful for the invention comprises two XTEN sequences, a first XTEN sequence inserted into a first XTEN insertion site and a second XTEN inserted into a second XTEN insertion site. Non-limiting examples of the first XTEN insertion site and the second XTEN insertion site are listed in Table 11.

TABLE 11. Exemplary Insertion Sites for Two XTENs

Insertion 1		Insertion 2	
Insertion Site	Domain	Insertion Site	Domain
745	B	2332	CT
26	A1	403	A2
40	A1	403	A2
18	A1	403	A2
26	A1	599	A2
40	A1	599	A2
18	A1	599	A2
1720	A3	1900	A3
1725	A3	1900	A3
1711	A3	1905	A3
1720	A3	1905	A3
1725	A3	1905	A3
1656	A3	26	A1
1656	A3	18	A1
1656	A3	40	A1
1656	A3	399	A2
1656	A3	403	A2
1656	A3	1725	A3
1656	A3	1720	A3
1900	A3	18	A1
1900	A3	26	A1
1900	A3	40	A1
1905	A3	18	A1
1905	A3	40	A1
1905	A3	26	A1
1910	A3	26	A1
18	A1	399	A2
26	A1	399	A2
40	A1	399	A2

Insertion 1		Insertion 2	
Insertion Site	Domain	Insertion Site	Domain
18	A1	403	A2
1656	A3	1900	A3
1656	A3	1905	A3
1711	A3	40	A1
1711	A3	26	A1
1720	A3	26	A1
1720	A3	40	A1
1720	A3	18	A1
1725	A3	26	A1
1725	A3	40	A1
1725	A3	18	A1
1720	A3	403	A2
1720	A3	399	A2
1711	A3	403	A2
1720	A3	403	A2
1725	A3	403	A2
1725	A3	399	A2
1711	A3	403	A2
1900	A3	399	A2
1900	A3	403	A2
1905	A3	403	A2
1905	A3	399	A2
1910	A3	403	A2

[0153] The two XTENs inserted or linked to the FVIII protein can be identical or different. In some embodiments, a FVIII protein useful for the invention comprises two XTEN sequences inserted in the FVIII protein, a first XTEN sequence inserted immediately downstream of amino acid 745 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately downstream of amino acid 2332 corresponding to SEQ ID NO: 65 (the C-terminus). In other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 18, 26, 40, 1656, or 1720 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately downstream of amino acid 403 corresponding to SEQ ID NO: 65. In yet other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 18, 26, or 40 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately downstream of amino acid 599 corresponding to SEQ ID NO: 65. In still other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately downstream of amino acid 18, 26, 40, 399, 403, 1725, 1720, 1900, 1905, or 2332 corresponding to SEQ ID NO: 65. In certain embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 1900 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately downstream of amino acid 18, 26, or 40 corresponding to SEQ ID NO: 65. In some embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 18, 26, or 40 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately downstream of amino acid 399 corresponding to SEQ ID NO: 65. In other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 1720 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately downstream of amino acid 18, 26, or 40 corresponding

to SEQ ID NO: 65. In still other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 1720 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately downstream of amino acid 18 corresponding to SEQ ID NO: 65. In a particular embodiment, the FVIII protein comprising two XTEN sequences, a first XTEN sequence inserted immediately downstream of amino acid 745 corresponding to SEQ ID NO: 65 and a second XTEN sequence inserted immediately downstream of amino acid 2332 corresponding to SEQ ID NO: 65, wherein the FVIII protein further has a deletion from amino acid 745 corresponding to SEQ ID NO: 65 to amino acid 1685 corresponding to SEQ ID NO: 65, a mutation or substitution at amino acid 1680 corresponding to SEQ ID NO: 65, e.g., Y1680F, a mutation or substitution at amino acid 1648 corresponding to SEQ ID NO: 65, *e.g.*, R1648A, or at least two mutations or substitutions at amino acid 1648 corresponding to SEQ ID NO: 65, *e.g.,* R1648A, and amino acid 1680 corresponding to SEQ ID NO: 65, *e.g.,* Y1680F. In a specific embodiment, the FVIII protein comprises two XTEN sequences, a first XTEN inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 65 and a second XTEN sequence inserted immediately downstream of amino acid 2332 of SEQ ID NO: 65, wherein the FVIII protein further has a deletion from amino acid 745 to amino acid 1656 corresponding to SEQ ID NO: 65.

[0154] In certain embodiments, a FVIII protein comprises three XTEN sequences, a first XTEN sequence inserted into a first XTEN insertion site, a second XTEN sequence inserted into a second XTEN sequence, and a third XTEN sequence inserted into a third XTEN insertion site. The first, second, or third XTEN sequences can be identical or different. The first, second, and third insertion sites can be selected from the group of any one of the insertion sites disclosed herein. In some embodiments, the FVIII protein comprising three XTEN sequences can further comprise a mutation or substitution, e.g., amino acid 1648 corresponding to SEQ ID NO: 65, *e.g.*, R1648A. For example, non-limiting examples of the first, second, and third XTEN insertion sites are listed in Table 12.

TABLE 12. Exemplary Insertion Sites for Three XTENs

Insertion 1		Insertion 2		Insertion 3	
Insertion Site	Domain	Insertion Site	Domain	Insertion Site	Domain
26	A1	403	A2	1656	A3
26	A1	403	A2	1720	A3
26	A1	403	A2	1900	A3
26	A1	1656	A3	1720	A3
26	A1	1656	A3	1900	A3
26	A1	1720	A3	1900	A3
403	A2	1656	A3	1720	A3
403	A2	1656	A3	1900	A3
403	A2	1720	A3	1900	A3
1656	A3	1720	A3	1900	A3
745	B	1900		2332	
18	A1	745	B	2332	CT
26	A1	745	B	2332	CT
40	A1	745	B	2332	CT
18	A1	745	B	2332	CT
40	A1	745	B	2332	CT
403	A2	745	B	2332	CT
399	A2	745	B	2332	CT
1725	A3	745	B	2332	CT
1720	A3	745	B	2332	CT
1711	A3	745	B	2332	CT

Insertion 1		Insertion 2		Insertion 3	
Insertion Site	Domain	Insertion Site	Domain	Insertion Site	Domain
1900	A3	745	B	2332	CT
1905	A3	745	B	2332	CT
1910	A3	745	B	2332	CT

[0155] In some embodiments, a FVIII protein comprises three XTEN sequences, a first XTEN sequence inserted immediately downstream of amino acid 26 corresponding to SEQ ID NO: 65, a second XTEN sequence inserted downstream of amino acid 403 corresponding to SEQ ID NO: 65, and a third XTEN sequence inserted downstream of amino acid 1656, 1720, or 1900 corresponding to SEQ ID NO: 65. In other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 26 corresponding to SEQ ID NO: 65, a second XTEN sequence is inserted downstream of amino acid 1656 corresponding to SEQ ID NO: 65, and a third XTEN sequence is inserted downstream of amino acid 1720 or 1900 corresponding to SEQ ID NO: 65. In yet other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 26 corresponding to SEQ ID NO: 65, a second XTEN sequence is inserted downstream of amino acid 1720 corresponding to SEQ ID NO: 65, and a third XTEN sequence is inserted downstream of amino acid 1900 corresponding to SEQ ID NO: 65. In still other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 403 corresponding to SEQ ID NO: 65, a second XTEN sequence is inserted downstream of amino acid 1656 corresponding to SEQ ID NO: 65, and a third XTEN sequence is inserted downstream of amino acid 1720 or 1900 corresponding to SEQ ID NO: 65. In other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 403 or 1656 corresponding to SEQ ID NO: 65, a second XTEN sequence is inserted downstream of amino acid 1720 corresponding to SEQ ID NO: 65, and a third XTEN sequence is inserted downstream of amino acid 1900 corresponding to SEQ ID NO: 65. In other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 18, 26, 40, 399, 403, 1711, 1720, 1725, 1900, 1905, or 1910 corresponding to SEQ ID NO: 65, a second XTEN sequence is inserted downstream of amino acid 745 corresponding to SEQ ID NO: 65, and a third XTEN sequence is inserted downstream of amino acid 2332 corresponding to SEQ ID NO: 65.

[0156] In other embodiments, a FVIII protein in the invention comprises four XTEN sequences, a first XTEN sequence inserted into a first insertion site, a second XTEN sequence inserted into a second insertion site, a third XTEN sequence inserted into a third insertion site, and a fourth XTEN sequence inserted into a fourth insertion site. The first, second, third, and fourth XTEN sequences can be identical, different, or combinations thereof. In some embodiments, the FVIII protein comprising four XTEN sequences can further comprise a mutation or substitution, *e.g.,* amino acid 1648 corresponding to SEQ ID NO: 65, *e.g.*, R1648A. Non-limiting examples of the first, second, third, and fourth XTEN insertion sites are listed in Table 13.

TABLE 13. Exemplary Insertion Sites for Four XTENs

Insertion 1		Insertion 2		Insertion 3		Insertion 4	
Insertion Site	Domain	Insertion Site	Domain	Insertion Site	Domain	Insertion Site	Domain
26	A1	403	A2	1656	a3	1720	A3
26	A1	403	A2	1656	a3	1900	A3
26	A1	403	A2	1720	A3	1900	A3
26	A1	1656	a3	1720	A3	1900	A3
403	A2	1656	a3	1720	A3	1900	A3
0040	A1	0403	A2	745	B	2332	CT
0040	A1	0403	A2	745	B	2332	CT
0018	A1	0409	A2	745	B	2332	CT
0040	A1	0409	A2	745	B	2332	CT

Insertion 1		Insertion 2		Insertion 3		Insertion 4	
Insertion Site	Domain	Insertion Site	Domain	Insertion Site	Domain	Insertion Site	Domain
0040	A1	0409	A2	745	B	2332	CT
0018	A1	0409	A2	745	B	2332	CT
0040	A1	1720	A3	745	B	2332	CT
0026	A1	1720	A3	745	B	2332	CT
0018	A1	1720	A3	745	B	2332	CT
0018	A1	1720	A3	745	B	2332	CT
0018	A1	1720	A3	745	B	2332	CT
0026	A1	1720	A3	745	B	2332	CT
0018	A1	1720	A3	745	B	2332	CT
0018	A1	1900	A3	745	B	2332	CT
0018	A1	1900	A3	745	B	2332	CT
0026	A1	1900	A3	745	B	2332	CT
0040	A1	1900	A3	745	B	2332	CT
0040	A1	1905	A3	745	B	2332	CT
0018	A1	1905	A3	745	B	2332	CT
0040	A1	1905	A3	745	B	2332	CT
0026	A1	1905	A3	745	B	2332	CT
0018	A1	1905	A3	745	B	2332	CT
0018	A1	1905	A3	745	B	2332	CT
0018	A1	1910	A3	745	B	2332	CT
0018	A1	1910	A3	745	B	2332	CT
0040	A1	1910	A3	745	B	2332	CT
0026	A1	1910	A3	745	B	2332	CT
0018	A1	1910	A3	745	B	2332	CT
0026	A1	1910	A3	745	B	2332	CT
0040	A1	1910	A3	745	B	2332	CT
0018	A1	1910	A3	745	B	2332	CT
0409	A2	1720	A3	745	B	2332	CT
0403	A2	1720	A3	745	B	2332	CT
0409	A2	1720	A3	745	B	2332	CT
0403	A2	1720	A3	745	B	2332	CT
0403	A2	1720	A3	745	B	2332	CT
0403	A2	1900	A3	745	B	2332	CT
0403	A2	1900	A3	745	B	2332	CT
0409	A2	1900	A3	745	B	2332	CT
0403	A2	1900	A3	745	B	2332	CT
0403	A2	1900	A3	745	B	2332	CT
0409	A2	1900	A3	745	B	2332	CT
0409	A2	1905	A3	745	B	2332	CT
0403	A2	1905	A3	745	B	2332	CT

Insertion 1		Insertion 2		Insertion 3		Insertion 4	
Insertion Site	Domain	Insertion Site	Domain	Insertion Site	Domain	Insertion Site	Domain
0403	A2	1905	A3	745	B	2332	CT
0403	A2	1905	A3	745	B	2332	CT
0409	A2	1905	A3	745	B	2332	CT
0403	A2	1905	A3	745	B	2332	CT
0409	A2	1910	A3	745	B	2332	CT
0403	A2	1910	A3	745	B	2332	CT
0403	A2	1910	A3	745	B	2332	CT
0403	A2	1910	A3	745	B	2332	CT
0403	A2	1910	A3	745	B	2332	CT
1720	A3	1900	A3	745	B	2332	CT
1720	A3	1905	A3	745	B	2332	CT
1720	A3	1910	A3	745	B	2332	CT
1720	A3	1910	A3	745	B	2332	CT
0403	A2	1656	a3	1720	A3	2332	CT
0403	A2	1656	a3	1900	A3	2332	CT
0403	A2	1720	A3	1900	A3	2332	CT
1656	a3	1720	A3	1900	A3	2332	CT
0018	A1	0403	A2	1656	a3	2332	CT
0018	A1	0403	A2	1720	A3	2332	CT
0018	A1	0403	A2	1900	A3	2332	CT
0018	A1	1656	a3	1720	A3	2332	CT
0018	A1	1656	a3	1900	A3	2332	CT
0018	A1	1720	A3	1900	A3	2332	CT
0018	A1	0403	A2	0745	B	2332	CT
0018	A1	0745	B	1720	A3	2332	CT
0018	A1	0745	B	1900	A3	2332	CT
0403	A2	0745	B	1720	A3	2332	CT
0403	A2	0745	B	1900	A3	2332	CT
0745	B	1720	A3	1900	A3	2332	CT
0188	A1	1900	A3	0745	B	2332	CT
0599		1900	A3	0745	B	2332	CT
2068		1900	A3	0745	B	2332	CT
2171		1900	A3	0745	B	2332	CT
2227		1900	A3	0745	B	2332	CT
2277		1900	A3	0745	B	2332	CT

[0157] In some embodiments, a FVIII protein comprises five XTEN sequences, a first XTEN sequence inserted into a first insertion site, a second XTEN sequence inserted into a second insertion site, a third XTEN sequence inserted into a third XTEN insertion site, a fourth XTEN sequence inserted into a fourth XTEN insertion site, and a fifth XTEN sequence inserted into a fifth XTEN insertion site. The first, second, third, fourth, of fifth XTEN

sequences can be identical, different, or combinations thereof. Non-limiting examples of the first, second, third, fourth, and fifth insertion sites are listed in Table 14.

TABLE 14. Exemplary Insertion Sites for Five XTENs

XTEN Insertion 1	XTEN insertion 2	XTEN Insertion 3	XTEN Insertion 4	XTEN Insertion 5
0403	1656	1720	1900	2332
0018	0403	1656	1720	2332
0018	0403	1656	1900	2332
0018	0403	1720	1900	2332
0018	1656	1720	1900	2332
0018	0403	0745	1720	2332
0018	0403	0745	1900	2332
0018	0745	1720	1900	2332
0403	0745	1720	1900	2332

[0158] In a particular example, a first XTEN is inserted between amino acids 26 and 27 corresponding to SEQ ID NO: 65, and a second XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65 (full-length mature FVIII). In another example, a first XTEN is inserted between amino acids 403 and 404 corresponding to SEQ ID NO: 65, and a second XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65. In some examples, a first XTEN is inserted between amino acids 1656 and 1657 corresponding to SEQ ID NO: 65, and a second XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65. In other examples, a first XTEN is inserted between amino acids 26 and 27 corresponding to SEQ ID NO: 65, a second XTEN is inserted between amino acids 1656 and 1657 corresponding to SEQ ID NO: 65, and a third XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65. In yet other embodiments, a first XTEN is inserted between amino acids 403 and 404 corresponding to SEQ ID NO: 65, a second XTEN is inserted between amino acids 1656 and 1657 corresponding to SEQ ID NO: 65, and a third XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65. In still other embodiments, a first XTEN is inserted between amino acids 403 and 404 corresponding to SEQ ID NO: 65, a second XTEN is inserted between amino acids 1656 and 1657 corresponding to SEQ ID NO: 65, and a third XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65. In certain embodiments, a first XTEN is inserted between amino acids 26 and 27 corresponding to SEQ ID NO: 65, a second XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65, and a third XTEN is inserted between amino acids 1900 and 1901 corresponding to SEQ ID NO: 65. In some embodiments, a first XTEN is inserted between amino acids 26 and 27 corresponding to SEQ ID NO: 65, a second XTEN is inserted between amino acids 1656 and 1657 corresponding to SEQ ID NO: 65, a third XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65, and a fourth XTEN is inserted between 1900 and 1901 corresponding to SEQ ID NO: 65.

[0159] In a particular embodiment, an XTEN sequence is inserted between amino acids 745 and 746 of a full-length Factor VIII or the corresponding insertion site of the B-domain deleted Factor VIII.

[0160] Disclosed herein are chimeric proteins comprising two polypeptide sequences, a first polypeptide sequence comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to a sequence selected from FVIII-161 (SEQ ID NO: 69), FVIII-169 (SEQ ID NO: 70), FVIII-170 (SEQ ID NO: 71), FVIII-173 (SEQ ID NO: 72); FVIII-195 (SEQ ID NO: 73); FVIII-196 (SEQ ID NO: 74), FVIII199 (SEQ ID NO: 75), FVIII-201 (SEQ ID NO: 76); FVIII-203 (SEQ ID NO: 77), FVIII-204 (SEQ ID NO: 78), FVIII-205 (SEQ ID NO: 79), FVIII-266 (SEQ ID NO: 80), FVIII-267 (SEQ ID NO: 81), FVIII-268 (SEQ ID NO: 82), FVIII-269 (SEQ ID NO: 83), FVIII-271 (SEQ ID NO: 84) or FVIII-272 (SEQ ID NO: 85) and a second polypeptide sequence comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to a sequence selected from VWF031 (SEQ ID NO: 86), VWF034 (SEQ ID NO: 87), or VWF-036.

II.D. Ig Constant Region or a portion thereof

[0161] Chimeric proteins disclosed herein also include two Ig constant regions or portions thereof, a first Ig constant region or a portion thereof fused to a FVIII protein by an optional linker and a second Ig constant region or a portion thereof fused to a VWF protein through the XTEN sequence having less than 288 amino acids. The Ig constant region or a portion thereof can improve pharmacokinetic or pharmacodynamic properties of the chimeric protein in combination with the XTEN sequence and the VWF protein. The Ig constant region or a portion thereof may extend a half-life of a molecule fused to the Ig constant region or a portion thereof.

[0162] An Ig constant region is comprised of domains denoted CH (constant heavy) domains (CH1, CH2, etc.). Depending on the isotype, (*i.e.* IgG, IgM, IgA, IgD, or IgE), the constant region can be comprised of three or four CH domains. Some isotypes *(e.g.* IgG) constant regions also contain a hinge region. *See* Janeway et al. 2001, Immunobiology, Garland Publishing, N.Y., N.Y.

[0163] An Ig constant region or a portion thereof for producing the chimeric protein of the present invention may be obtained from a number of different sources. An Ig constant region or a portion thereof may be derived from a human Ig. It is understood, however, that the Ig constant region or a portion thereof may be derived from an Ig of another mammalian species, including for example, a rodent *(e.g.* a mouse, rat, rabbit, guinea pig) or non-human primate (*e.g.* chimpanzee, macaque) species. Moreover, the Ig constant region or a portion thereof may be derived from any Ig class, including IgM, IgG, IgD, IgA, and IgE, and any Ig isotype, including IgG1, IgG2, IgG3, and IgG4. Specifically, the human isotype IgG1 is used.

[0164] A variety of the Ig constant region gene sequences (e.g., human constant region gene sequences) are available in the form of publicly accessible deposits. Constant region domains sequence can be selected having a particular effector function (or lacking a particular effector function) or with a particular modification to reduce immunogenicity. Many sequences of antibodies and antibody-encoding genes have been published and suitable Ig constant region sequences (e.g., hinge, CH2, and/or CH3 sequences, or portions thereof) can be derived from these sequences using art recognized techniques. The genetic material obtained using any of the foregoing methods may then be altered or synthesized to obtain polypeptides of the present invention. It will further be appreciated that the scope of this invention encompasses alleles, variants and mutations of constant region DNA sequences.

[0165] The sequences of the Ig constant region or a portion thereof can be cloned, e.g., using the polymerase chain reaction and primers which are selected to amplify the domain of interest. To clone a sequence of the Ig constant region or a portion thereof from an antibody, mRNA can be isolated from hybridoma, spleen, or lymph cells, reverse transcribed into DNA, and antibody genes amplified by PCR. PCR amplification methods are described in detail in U.S. Pat. Nos. 4,683,195; 4,683,202; 4,800,159; 4,965,188; and in, *e.g.,* "PCR Protocols: A Guide to Methods and Applications" Innis et al. eds., Academic Press, San Diego, CA (1990); Ho et al. 1989. Gene 77:51; Horton et al. 1993. Methods Enzymol. 217:270). PCR may be initiated by consensus constant region primers or by more specific primers based on the published heavy and light chain DNA and amino acid sequences. As discussed above, PCR also may be used to isolate DNA clones encoding the antibody light and heavy chains. In this case the libraries may be screened by consensus primers or larger homologous probes, such as mouse constant region probes. Numerous primer sets suitable for amplification of antibody genes are known in the art (*e.g.*, 5' primers based on the N-terminal sequence of purified antibodies (Benhar and Pastan. 1994. Protein Engineering 7:1509); rapid amplification of cDNA ends (Ruberti, F. et al. 1994. J. Immunol. Methods 173:33); antibody leader sequences (Larrick et al. 1989 Biochem. Biophys. Res. Commun. 160:1250). The cloning of antibody sequences is further described in Newman et al., U.S. Pat. No. 5,658,570, filed January 25, 1995.

[0166] An Ig constant region used herein can include all domains and the hinge region or portions thereof. The

Ig constant region or a portion thereof may comprise CH2 domain, CH3 domain, and a hinge region, *i.e.,* an Fc region or an FcRn binding partner.

[0167] As used herein, the term "Fc region" is defined as the portion of a polypeptide which corresponds to the Fc region of native Ig, *i.e.,* as formed by the dimeric association of the respective Fc domains of its two heavy chains. A native Fc region forms a homodimer with another Fc region. In contrast, the term "genetically-fused Fc region" or "single-chain Fc region" (scFc region), as used herein, refers to a synthetic dimeric Fc region comprised of Fc domains genetically linked within a single polypeptide chain (*i.e.*, encoded in a single contiguous genetic sequence).

[0168] In one embodiment, the "Fc region" refers to the portion of a single Ig heavy chain beginning in the hinge region just upstream of the papain cleavage site (*i.e.* residue 216 in IgG, taking the first residue of heavy chain constant region to be 114) and ending at the C-terminus of the antibody. Accordingly, a complete Fc domain comprises at least a hinge domain, a CH2 domain, and a CH3 domain.

[0169] The Fc region of an Ig constant region, depending on the Ig isotype can include the CH2, CH3, and CH4 domains, as well as the hinge region. Chimeric proteins comprising an Fc region of an Ig bestow several desirable properties on a chimeric protein including increased stability, increased serum half-life (see Capon et al., 1989, Nature 337:525) as well as binding to Fc receptors such as the neonatal Fc receptor (FcRn) (U.S. Pat. Nos. 6,086,875, 6,485,726, 6,030,613; WO 03/077834; US2003-0235536A1).

[0170] An Ig constant region or a portion thereof can be an FcRn binding partner. FcRn is active in adult epithelial tissues and expressed in the lumen of the intestines, pulmonary airways, nasal surfaces, vaginal surfaces, colon and rectal surfaces (U.S. Pat. No. 6,485,726). An FcRn binding partner is a portion of an Ig that binds to FcRn.

[0171] The FcRn receptor has been isolated from several mammalian species including humans. The sequences of the human FcRn, monkey FcRn, rat FcRn, and mouse FcRn are known (Story et al. 1994, J. Exp. Med. 180:2377). The FcRn receptor binds IgG (but not other Ig classes such as IgA, IgM, IgD, and IgE) at relatively low pH, actively transports the IgG transcellularly in a luminal to serosal direction, and then releases the IgG at relatively higher pH found in the interstitial fluids. It is expressed in adult epithelial tissue (U.S. Pat. Nos. 6,485,726, 6,030,613, 6,086,875; WO 03/077834; US2003-0235536A1) including lung and intestinal epithelium (Israel et al. 1997, Immunology 92:69) renal proximal tubular epithelium (Kobayashi et al. 2002, Am. J. Physiol. Renal Physiol. 282:F358) as well as nasal epithelium, vaginal surfaces, and biliary tree surfaces.

[0172] FcRn binding partners useful in the present invention encompass molecules that can be specifically bound by the FcRn receptor including whole IgG, the Fc fragment of IgG, and other fragments that include the complete binding region of the FcRn receptor. The region of the Fc portion of IgG that binds to the FcRn receptor has been described based on X-ray crystallography (Burmeister et al. 1994, Nature 372:379). The major contact area of the Fc with the FcRn is near the junction of the CH2 and CH3 domains. Fc-FcRn contacts are all within a single Ig heavy chain. The FcRn binding partners include whole IgG, the Fc fragment of IgG, and other fragments of IgG that include the complete binding region of FcRn. The major contact sites include amino acid residues 248, 250-257, 272, 285, 288, 290-291, 308-311, and 314 of the CH2 domain and amino acid residues 385-387, 428, and 433-436 of the CH3 domain. References made to amino acid numbering of Igs or Ig fragments, or regions, are all based on Kabat et al. 1991, Sequences of Proteins of Immunological Interest, U.S. Department of Public Health, Bethesda, Md.

[0173] Fc regions or FcRn binding partners bound to FcRn can be effectively shuttled across epithelial barriers by FcRn, thus providing a non-invasive means to systemically administer a desired therapeutic molecule. Additionally, fusion proteins comprising an Fc region or an FcRn binding partner are endocytosed by cells expressing the FcRn. But instead of being marked for degradation, these fusion proteins are recycled out into circulation again, thus increasing the *in vivo* half-life of these proteins. The portions of Ig constant regions may

be an Fc region or an FcRn binding partner that typically associates, via disulfide bonds and other non-specific interactions, with another Fc region or another FcRn binding partner to form dimers and higher order multimers.

[0174] Two FcRn receptors can bind a single Fc molecule. Crystallographic data suggest that each FcRn molecule binds a single polypeptide of the Fc homodimer. In one embodiment, linking the FcRn binding partner, e.g., an Fc fragment of an IgG, to a biologically active molecule provides a means of delivering the biologically active molecule orally, buccally, sublingually, rectally, vaginally, as an aerosol administered nasally or via a pulmonary route, or via an ocular route. In another embodiment, the chimeric protein can be administered invasively, e.g., subcutaneously, intravenously.

[0175] An FcRn binding partner region is a molecule or a portion thereof that can be specifically bound by the FcRn receptor with consequent active transport by the FcRn receptor of the Fc region. Specifically bound refers to two molecules forming a complex that is relatively stable under physiologic conditions. Specific binding is characterized by a high affinity and a low to moderate capacity as distinguished from nonspecific binding which usually has a low affinity with a moderate to high capacity. Typically, binding is considered specific when the affinity constant KA is higher than 10^6 M^{-1}, or higher than 10^8 M^{-1}. If necessary, non-specific binding can be reduced without substantially affecting specific binding by varying the binding conditions. The appropriate binding conditions such as concentration of the molecules, ionic strength of the solution, temperature, time allowed for binding, concentration of a blocking agent *(e.g.* serum albumin, milk casein), etc., may be optimized by a skilled artisan using routine techniques.

[0176] In certain embodiments, a chimeric protein of the invention comprises one or more truncated Fc regions that are nonetheless sufficient to confer Fc receptor (FcR) binding properties to the Fc region. For example, the portion of an Fc region that binds to FcRn *(i.e.,* the FcRn binding portion) comprises from about amino acids 282-438 of IgG1, EU numbering (with the primary contact sites being amino acids 248, 250-257, 272, 285, 288, 290-291, 308-311, and 314 of the CH2 domain and amino acid residues 385-387, 428, and 433-436 of the CH3 domain. Thus, an Fc region of the invention may comprise or consist of an FcRn binding portion. FcRn binding portions may be derived from heavy chains of any isotype, including IgG1, IgG2, IgG3 and IgG4. In one embodiment, an FcRn binding portion from an antibody of the human isotype IgG1 is used. In another embodiment, an FcRn binding portion from an antibody of the human isotype IgG4 is used.

[0177] In another embodiment, the "Fc region" includes an amino acid sequence of an Fc domain or derived from an Fc domain. In certain embodiments, an Fc region comprises at least one of: a hinge (e.g., upper, middle, and/or lower hinge region) domain (about amino acids 216-230 of an antibody Fc region according to EU numbering), a CH2 domain (about amino acids 231-340 of an antibody Fc region according to EU numbering), a CH3 domain (about amino acids 341-438 of an antibody Fc region according to EU numbering), a CH4 domain, or a variant, portion, or fragment thereof. In other embodiments, an Fc region comprises a complete Fc domain *(i.e.,* a hinge domain, a CH2 domain, and a CH3 domain). In some embodiments, an Fc region comprises, consists essentially of, or consists of a hinge domain (or a portion thereof) fused to a CH3 domain (or a portion thereof), a hinge domain (or a portion thereof) fused to a CH2 domain (or a portion thereof), a CH2 domain (or a portion thereof) fused to a CH3 domain (or a portion thereof), a CH2 domain (or a portion thereof) fused to both a hinge domain (or a portion thereof) and a CH3 domain (or a portion thereof). In still other embodiments, an Fc region lacks at least a portion of a CH2 domain (e.g., all or part of a CH2 domain). In a particular embodiment, an Fc region comprises or consists of amino acids corresponding to EU numbers 221 to 447.

[0178] The Fc regions denoted as F, F1, or F2 herein may be obtained from a number of different sources. In one embodiment, an Fc region of the polypeptide is derived from a human Ig. It is understood, however, that an Fc region may be derived from an Ig of another mammalian species, including for example, a rodent *(e.g.* a mouse, rat, rabbit, or guinea pig) or non-human primate *(e.g.* chimpanzee, macaque) species. Moreover, the polypeptide of the Fc domains or portions thereof may be derived from any Ig class, including IgM, IgG, IgD, IgA and IgE, and any Ig isotype, including IgG1, IgG2, IgG3 and IgG4. In another embodiment, the human isotype IgG1 is used.

[0179] In certain embodiments, the Fc variant confers a change in at least one effector function imparted by an Fc region comprising said wild-type Fc domain (e.g., an improvement or reduction in the ability of the Fc region to bind to Fc receptors (*e.g.* FcγRI, FcγRII, or FcγRIII) or complement proteins *(e.g.* C1q), or to trigger antibody-dependent cytotoxicity (ADCC), phagocytosis, or complement-dependent cytotoxicity (CDCC)). In other embodiments, the Fc variant provides an engineered cysteine residue.

[0180] The Fc regions of the invention may employ art-recognized Fc variants which are known to impart a change *(e.g.,* an enhancement or reduction) in effector function and/or FcR or FcRn binding. Specifically, a binding molecule of the invention may include, for example, a change *(e.g.,* a substitution) at one or more of the amino acid positions disclosed in International PCT Publications WO88/07089A1, WO96/14339A1, WO98/05787A1, WO98/23289A1, WO99/51642A1, WO99/58572A1, WO00/09560A2, WO00/32767A1, WO00/42072A2, WO02/44215A2, WO02/060919A2, WO03/074569A2, WO04/016750A2, WO04/029207A2, WO04/035752A2, WO04/063351A2, WO04/074455A2, WO04/099249A2, WO05/040217A2, WO04/044859, WO05/070963A1, WO05/077981A2, WO05/092925A2, WO05/123780A2, WO06/019447A1, WO06/047350A2, and WO06/085967A2; US Patent Publication Nos. US2007/0231329, US2007/0231329, US2007/0237765, US2007/0237766, US2007/0237767, US2007/0243188, US20070248603, US20070286859, US20080057056 ; or US Patents 5,648,260; 5,739,277; 5,834,250; 5,869,046; 6,096,871; 6,121,022; 6,194,551; 6,242,195; 6,277,375; 6,528,624; 6,538,124; 6,737,056; 6,821,505; 6,998,253; 7,083,784; 7,404,956, and 7,317,091. In one embodiment, the specific change (e.g., the specific substitution of one or more amino acids disclosed in the art) may be made at one or more of the disclosed amino acid positions. In another embodiment, a different change at one or more of the disclosed amino acid positions (e.g., the different substitution of one or more amino acid position disclosed in the art) may be made.

[0181] The Fc region or FcRn binding partner of IgG can be modified according to well recognized procedures such as site directed mutagenesis and the like to yield modified IgG or Fc fragments or portions thereof that will be bound by FcRn. Such modifications include modifications remote from the FcRn contact sites as well as modifications within the contact sites that preserve or even enhance binding to the FcRn. For example, the following single amino acid residues in human IgG1 Fc (Fc y1) can be substituted without significant loss of Fc binding affinity for FcRn: P238A, S239A, K246A, K248A, D249A, M252A, T256A, E258A, T260A, D265A, S267A, H268A, E269A, D270A, E272A, L274A, N276A, Y278A, D280A, V282A, E283A, H285A, N286A, T289A, K290A, R292A, E293A, E294A, Q295A, Y296F, N297A, S298A, Y300F, R301A, V303A, V305A, T307A, L309A, Q311A, D312A, N315A, K317A, E318A, K320A, K322A, S324A, K326A, A327Q, P329A, A330Q, P331A, E333A, K334A, T335A, S337A, K338A, K340A, Q342A, R344A, E345A, Q347A, R355A, E356A, M358A, T359A, K360A, N361A, Q362A, Y373A, S375A, D376A, A378Q, E380A, E382A, S383A, N384A, Q386A, E388A, N389A, N390A, Y391F, K392A, L398A, S400A, D401A, D413A, K414A, R416A, Q418A, Q419A, N421A, V422A, S424A, E430A, N434A, T437A, Q438A, K439A, S440A, S444A, and K447A, where for example P238A represents wild type proline substituted by alanine at position number 238. As an example, a specific embodiment incorporates the N297A mutation, removing a highly conserved N-glycosylation site. In addition to alanine other amino acids may be substituted for the wild type amino acids at the positions specified above. Mutations may be introduced singly into Fc giving rise to more than one hundred Fc regions distinct from the native Fc. Additionally, combinations of two, three, or more of these individual mutations may be introduced together, giving rise to hundreds more Fc regions. Moreover, one of the Fc region of a construct of the invention may be mutated and the other Fc region of the construct not mutated at all, or they both may be mutated but with different mutations.

[0182] Certain of the above mutations may confer new functionality upon the Fc region or FcRn binding partner. For example, one embodiment incorporates N297A, removing a highly conserved N-glycosylation site. The effect of this mutation is to reduce immunogenicity, thereby enhancing circulating half-life of the Fc region, and to render the Fc region incapable of binding to FcγRI, FcγRIIA, FcγRIIB, and FcγRIIIA, without compromising affinity for FcRn (Routledge et al. 1995, Transplantation 60:847; Friend et al. 1999, Transplantation 68:1632; Shields et al. 1995, J. Biol. Chem. 276:6591). As a further example of new functionality arising from mutations described above affinity for FcRn may be increased beyond that of wild type in some instances. This increased affinity may reflect an increased "on" rate, a decreased "off" rate or both an increased "on" rate and a decreased

"off' rate. Examples of mutations believed to impart an increased affinity for FcRn include, but not limited to, T256A, T307A, E380A, and N434A (Shields et al. 2001, J. Biol. Chem. 276:6591).

[0183] Additionally, at least three human Fc gamma receptors appear to recognize a binding site on IgG within the lower hinge region, generally amino acids 234-237. Therefore, another example of new functionality and potential decreased immunogenicity may arise from mutations of this region, as for example by replacing amino acids 233-236 of human IgG1 "ELLG" to the corresponding sequence from IgG2 "PVA" (with one amino acid deletion). It has been shown that FcγRI, FcγRII, and FcγRIII, which mediate various effector functions will not bind to IgG1 when such mutations have been introduced. Ward and Ghetie 1995, Therapeutic Immunology 2:77 and Armour et al. 1999, Eur. J. Immunol. 29:2613.

[0184] The Ig constant region or a portion thereof, e.g., an Fc region, may be a polypeptide including the sequence PKNSSMISNTP (SEQ ID NO: 89 or SEQ ID NO: 3 of U.S. Pat. No. 5,739,277) and optionally further including a sequence selected from HQSLGTQ (SEQ ID NO: 90), HQNLSDGK (SEQ ID NO: 91), HQNISDGK (SEQ ID NO: 92), or VISSHLGQ (SEQ ID NO: 93) (or SEQ ID NOs: 11, 1, 2, and 31, respectively of U.S. Pat. No. 5,739,277).

[0185] The immunoglobulin constant region or a portion thereof may comprise an amino acid sequence in the hinge region or a portion thereof that forms one or more disulfide bonds with another immunoglobulin constant region or a portion thereof. The disulfide bond by the immunoglobulin constant region or a portion thereof places the first polypeptide comprising FVIII and the second polypeptide comprising the VWF fragment together so that endogenous VWF does not replace the VWF fragment and does not bind to the FVIII. Therefore, the disulfide bond between the first immunoglobulin constant region or a portion thereof and a second immunoglobulin constant region or a portion thereof prevents interaction between endogenous VWF and the FVIII protein. This inhibition of interaction between the VWF and the FVIII protein allows the half-life of the chimeric protein to go beyond the two fold limit. The hinge region or a portion thereof can further be linked to one or more domains of CH1, CH2, CH3, a fragment thereof, and any combinations thereof. In particular, the immunoglobulin constant region or a portion thereof may be a hinge region and CH2.

[0186] The Ig constant region or a portion thereof may be hemi-glycosylated. For example, the chimeric protein comprising two Fc regions or FcRn binding partners may contain a first, glycosylated, Fc region *(e.g.,* a glycosylated CH2 region) or FcRn binding partner and a second, aglycosylated, Fc region *(e.g.,* an aglycosylated CH2 region) or FcRn binding partner. In one embodiment, a linker may be interposed between the glycosylated and aglycosylated Fc regions. In another embodiment, the Fc region or FcRn binding partner is fully glycosylated, *i.e.,* all of the Fc regions are glycosylated. In other embodiments, the Fc region may be aglycosylated, *i.e.,* none of the Fc moieties are glycosylated.

[0187] A chimeric protein as disclosed may comprise an amino acid substitution to an Ig constant region or a portion thereof *(e.g.,* Fc variants), which alters the antigen-independent effector functions of the Ig constant region, in particular the circulating half-life of the protein.

[0188] Such proteins exhibit either increased or decreased binding to FcRn when compared to proteins lacking these substitutions and, therefore, have an increased or decreased half-life in serum, respectively. Fc variants with improved affinity for FcRn are anticipated to have longer serum half-lives, and such molecules have useful applications in methods of treating mammals where long half-life of the administered polypeptide is desired, *e.g.,* to treat a chronic disease or disorder (*see, e.g.,* US Patents 7,348,004, 7,404,956, and 7,862,820). In contrast, Fc variants with decreased FcRn binding affinity are expected to have shorter half-lives, and such molecules are also useful, for example, for administration to a mammal where a shortened circulation time may be advantageous, e.g. for in vivo diagnostic imaging or in situations where the starting polypeptide has toxic side effects when present in the circulation for prolonged periods. Fc variants with decreased FcRn binding affinity are also less likely to cross the placenta and, thus, are also useful in the treatment of diseases or disorders in pregnant women. In addition, other applications in which reduced FcRn binding affinity may be desired include

those applications in which localization the brain, kidney, and/or liver is desired. In one exemplary embodiment, the chimeric protein of the invention exhibit reduced transport across the epithelium of kidney glomeruli from the vasculature. In another embodiment, the chimeric protein of the invention exhibit reduced transport across the blood brain barrier (BBB) from the brain, into the vascular space. In one embodiment, a protein with altered FcRn binding comprises at least one Fc region or FcRn binding partner (e.g, one or two Fc regions or FcRn binding partners) having one or more amino acid substitutions within the "FcRn binding loop" of an Ig constant region. The FcRn binding loop is comprised of amino acid residues 280-299 (according to EU numbering) of a wild-type, full-length, Fc region. An Ig constant region or a portion thereof in a chimeric protein as disclosed having altered FcRn binding affinity may comprise at least one Fc region or FcRn binding partner having one or more amino acid substitutions within the 15 Á FcRn "contact zone." As used herein, the term 15 Á FcRn "contact zone" includes residues at the following positions of a wild-type, full-length Fc moiety: 243-261, 275-280, 282-293, 302-319, 336- 348, 367, 369, 372-389, 391, 393, 408, 424, 425-440 (EU numbering). An Ig constant region or a portion thereof as disclosed having altered FcRn binding affinity may comprise at least one Fc region or FcRn binding partner having one or more amino acid substitutions at an amino acid position corresponding to any one of the following EU positions: 256, 277-281, 283-288, 303-309, 313, 338, 342, 376, 381, 384, 385, 387, 434 (*e.g.,* N434A or N434K), and 438. Exemplary amino acid substitutions which altered FcRn binding activity are disclosed in International PCT Publication No. WO05/047327.

[0189] An Fc region or FcRn binding partner used in the invention may also comprise an art recognized amino acid substitution which alters the glycosylation of the chimeric protein. For example, the Fc region or FcRn binding partner of the chimeric protein linked to a VWF fragment or a FVIII protein may comprise an Fc region having a mutation leading to reduced glycosylation (e.g., N- or O-linked glycosylation) or may comprise an altered glycoform of the wild-type Fc moiety (e.g., a low fucose or fucose-free glycan).

[0190] An unprocessed chimeric protein may comprise a genetically fused Fc region (*i.e.*, scFc region) having two or more of its constituent Ig constant region or a portion thereof independently selected from the Ig constant region or a portion thereof described herein. In one embodiment, the Fc regions of a dimeric Fc region are the same. In another embodiment, at least two of the Fc regions are different. For example, the Fc regions or FcRn binding partners of the proteins of the invention comprise the same number of amino acid residues or they may differ in length by one or more amino acid residues (e.g., by about 5 amino acid residues (e.g., 1, 2, 3, 4, or 5 amino acid residues), about 10 residues, about 15 residues, about 20 residues, about 30 residues, about 40 residues, or about 50 residues). In yet other embodiments, the Fc regions or FcRn binding partners of the protein of the invention may differ in sequence at one or more amino acid positions. For example, at least two of the Fc regions or FcRn binding partners may differ at about 5 amino acid positions (e.g., 1, 2, 3, 4, or 5 amino acid positions), about 10 positions, about 15 positions, about 20 positions, about 30 positions, about 40 positions, or about 50 positions).

II.E. Linkers

[0191] The chimeric protein of the present invention further comprises one or more linkers. One type of the linkers is a cleavable linker, which can be cleaved by various proteases when administered to a subject in vivo, *e.g.,* at a site of coagulation. The cleavable linker can allow cleavage of moiety, *e.g.,* a VWF protein, from the XTEN sequence, thus from the chimeric protein at the site of the coagulation cascade, thereby allowing activated FVIII (FVIIIa) to have its FVIIIa activity. Another type of the linkers is a processable linker, which contains an intracellular cleavage site and thus can be cleaved by an intracellular processing enzyme in a host cell, allowing convenient expression of a polypeptide and formation of a chimeric protein.

[0192] One or more linkers can be present between any two proteins in the chimeric protein. The chimeric protein according to the invention comprises a first polypeptide which comprises (i) a FVIII protein and (ii) a first Fc region thereof and a second polypeptide which comprises (iii) a VWF fragment, (iv) a cleavable linker, (v) an XTEN sequence, and (vi) a second Fc region. In some embodiments, the first polypeptide further comprises a

linker, e.g., a cleavable linker between the FVIII protein and the first Fc region.

[0193] The chimeric protein may comprise a single chain comprising (i) a FVIII protein, (ii) a first Ig constant region or a portion thereof, (iii) a linker (e.g., a processable linker), (iv) a VWF protein, (v) an XTEN sequence, and (vi) a second Ig constant region or a portion thereof. A chimeric protein may comprise a single chain comprising (i) a FVIII protein, (ii) a first Ig constant region or a portion thereof, (iii) a first linker (e.g., a processable linker), (iv) a VWF protein, (v) a second linker (e.g., a cleavable linker), (vi) an XTEN sequence, and (vii) a second Ig constant region or a portion thereof. The processable linker can be processed after the chimeric protein is expressed in the host cell; thus the chimeric protein produced in the host cell can be in the final form comprising two or three polypeptide chains.

[0194] The linker can comprise any organic molecule. In one embodiment, the linker comprises a polymer, e.g., polyethylene glycol (PEG) or hydroxyethyl starch (HES). In another embodiment, the linker comprises an amino acid sequence. The linker can comprise at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 400 ,500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 amino acids. The linker can comprise 1-5 amino acids, 1-10 amino acids, 1-20 amino acids, 10-50 amino acids, 50-100 amino acids, 100-200 amino acids, 200-300 amino acids, 300-400 amino acids, 400-500 amino acids, 500-600 amino acids, 600-700 amino acids, 700-800 amino acids, 800-900 amino acids, or 900-1000 amino acids. In one embodiment, the linker comprises an XTEN sequence. Additional examples of XTEN can be used according to the present invention and are disclosed in US Patent Publication Nos. 2010/0239554 A1, 2010/0323956 A1, 2011/0046060 A1, 2011/0046061 A1, 2011/0077199 A1, or 2011/0172146 A1, or International Patent Publication Nos. WO 2010091122 A1, WO 2010144502 A2, WO 2010144508 A1, WO 2011028228 A1, WO 2011028229 A1, or WO 2011028344 A2. In another embodiment, the linker is a PAS sequence.

[0195] In one embodiment, the linker is a polymer, e.g., polyethylene glycol (PEG) or hydroxyethyl starch (HES). In another embodiment, the linker is an amino acid sequence. The linker can comprise at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 amino acids. The linker can comprise 1-5 amino acids, 1-10 amino acids, 1-20 amino acids, 10-50 amino acids, 50-100 amino acids, 100-200 amino acids, 200-300 amino acids, 300-400 amino acids, 400-500 amino acids, 500-600 amino acids, 600-700 amino acids, 700-800 amino acids, 800-900 amino acids, or 900-1000 amino acids.

[0196] Examples of linkers are well known in the art. In one embodiment, the linker comprises the sequence G_n. The linker can comprise the sequence $(GA)_n$. The linker can comprise the sequence $(GGS)_n$. In other embodiments, the linker comprises $(GGGS)_n$(SEQ ID NO: 101). In still other embodiments, the linker comprises the sequence $(GGS)_n(GGGGS)_n$(SEQ ID NO: 95). In these instances, n may be an integer from 1-100. In other instances, n may be an integer from 1-20, *i.e.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. Examples of linkers include, but are not limited to, GGG, SGGSGGS (SEQ ID NO: 96), GGSGGSGGSGGSGGG (SEQ ID NO: 97), GGSGGSGGGGSGGGGS (SEQ ID NO: 98), GGSGGSGGSGGSGGSGGS (SEQ ID NO: 99), or GGGGSGGGGSGGGGS (SEQ ID NO: 100). The linker does not eliminate or diminish the VWF protein activity or the clotting activity of Factor VIII. Optionally, the linker enhances the VWF protein activity or the clotting activity of Factor VIII protein, e.g., by further diminishing the effects of steric hindrance and making the VWF protein or Factor VIII portion more accessible to its target binding site.

[0197] In one embodiment, the linker useful for the chimeric protein is 15-25 amino acids long. In another embodiment, the linker useful for the chimeric protein is 15-20 amino acids long. In some embodiments, the linker for the chimeric protein is 10-25 amino acids long. In other embodiments, the linker for the chimeric protein is 15 amino acids long. In still other embodiments, the linker for the chimeric protein is $(GGGGS)_n$ (SEQ ID NO: 94) where G represents glycine, S represents serine and n is an integer from 1-20.

II. F. Cleavage Sites

[0198] A cleavable linker can incorporate a moiety capable of being cleaved either chemically (e.g., hydrolysis of an ester bond), enzymatically (*i.e.*, incorporation of a protease cleavage sequence), or photolytically (e.g., a chromophore such as 3-amino-3-(2-nitrophenyl) proprionic acid (ANP)) in order to release one molecule from another.

[0199] In one embodiment, a cleavable linker comprises one or more cleavage sites at the N-terminus or C-terminus or both. In another embodiment, the cleavable linker consists essentially of or consists of one or more cleavable sites. In other embodiments, the cleavable linker comprises heterologous amino acid linker sequences described herein or polymers and one or more cleavable sites.

[0200] In certain embodiments, a cleavable linker comprises one or more cleavage sites that can be cleaved in a host cell (*i.e.*, intracellular processing sites). Non limiting examples of the cleavage site include RRRR (SEQ ID NO: 102), RKRRKR (SEQ ID NO: 103), and RRRRS (SEQ ID NO: 104).

[0201] In some embodiments, a cleavable linker comprises an a1 region from FVIII, an a2 region from FVIII, an a3 region from FVIII, a thrombin cleavable site which comprises X-V-P-R (SEQ ID NO: 105) and a PARI exosite interaction motif, wherein X is an aliphatic amino acid, or any combinations thereof. In one embodiment, a cleavable linker comprises the a2 region which comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to Glu720 to Arg740 corresponding to full-length FVIII, wherein the a2 region is capable of being cleaved by thrombin. In a particular embodiment, a cleavable linker comprises an a2 region which comprises ISDKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 106). In other embodiments, a cleavable linker comprises the a1 region which comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to Met337 to Arg372 corresponding to full-length FVIII, wherein the a1 region is capable of being cleaved by thrombin. In a particular embodiment, the a1 region comprises ISMKNNEEAEDYDDDLTDSEMDVVRFDDDNSPSFIQIRSV (SEQ ID NO: 107). In some embodiments, a cleavable linker of the invention comprises the a3 region which comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to Glu1649 to Arg1689 corresponding to full-length FVIII, wherein the a3 region is capable of being cleaved by thrombin. In a specific embodiment, a cleavable linker for the invention comprises an a3 region which comprises ISEITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQ (SEQ ID NO: 108).

[0202] In other embodiments, a cleavable linker comprises the thrombin cleavage site which comprises X-V-P-R (SEQ ID NO: 105) and the PARI exosite interaction motif and wherein the PARI exosite interaction motif comprises S-F-L-L-R-N (SEQ ID NO: 109). The PARI exosite interaction motif can further comprise an amino acid sequence selected from P, P-N, P-N-D, P-N-D-K (SEQ ID NO: 110), P-N-D-K-Y (SEQ ID NO: 111), P-N-D-K-Y-E (SEQ ID NO: 112), P-N-D-K-Y-E-P (SEQ ID NO: 113), P-N-D-K-Y-E-P-F (SEQ ID NO: 114), P-N-D-K-Y-E-P-F-W (SEQ ID NO: 115), P-N-D-K-Y-E-P-F-W-E (SEQ ID NO: 116), P-N-D-K-Y-E-P-F-W-E-D (SEQ ID NO: 117), P-N-D-K-Y-E-P-F-W-E-D-E (SEQ ID NO: 118), P-N-D-K-YE-P-F-W-E-D-E-E (SEQ ID NO: 119), P-N-D-K-Y-E-P-F-W-E-D-E-E-S (SEQ ID NO: 120), or any combination thereof. In some embodiments, the aliphatic amino acid is selected from Glycine, Alanine, Valine, Leucine, or Isoleucine.

[0203] In other embodiments, a cleavable linker comprises one or more cleavage sites that are cleaved by a protease after a chimeric protein comprising the cleavable linker is administered to a subject. In one embodiment, the cleavage site is cleaved by a 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, MMP-13, MMP-17, and MMP-20. In another embodiment, the cleavage site is selected from the group consisting of a FXIa cleavage site (*e.g.,* KLTR↓AET (SEQ ID NO: 121)), a FXIa cleavage site (*e.g,* DFTR↓VVG (SEQ ID NO: 122)), a FXIIa cleavage site (e.g., TMTR↓IVGG (SEQ ID NO: 123)), a Kallikrein cleavage site (*e.g.,* SPFR↓STGG (SEQ ID NO: 124)), a FVIIa cleavage site (*e.g.,* LQVR↓IVGG (SEQ ID NO: 125)), a FIXa cleavage site (*e.g.,* PLGR↓IVGG (SEQ ID NO: 126)), a FXa cleavage site (*e.g.,* IEGR↓TVGG (SEQ ID NO: 127)), a FIIa (thrombin) cleavage site

(*e.g*, LTPR↓SLLV (SEQ ID NO: 128)), a Elastase-2 cleavage site (*e.g.,* LGPV↓SGVP (SEQ ID NO: 129)), a Granzyme-B cleavage (*e.g.,* VAGD↓SLEE (SEQ ID NO: 130)), a MMP-12 cleavage site (*e.g.,* GPAG↓LGGA (SEQ ID NO: 131)), a MMP-13 cleavage site *(e.g.,* GPAG↓LRGA (SEQ ID NO: 132)), a MMP-17 cleavage site (*e.g.*, APLG↓LRLR (SEQ ID NO: 133)), a MMP-20 cleavage site (*e.g.,* PALP↓LVAQ (SEQ ID NO: 134)), a TEV cleavage site (*e.g.,* ENLYFQ↓G (SEQ ID NO: 135)), a Enterokinase cleavage site (*e.g.*, DDDK↓IVGG (SEQ ID NO: 136)), a Protease 3C (PRESCISSION™) cleavage site (*e.g.*, LEVLFQ↓GP (SEQ ID NO: 137)), and a Sortase A cleavage site (*e.g.*, LPKT↓GSES) (SEQ ID NO: 138). In certain embodiments, the FXIa cleavage sites include, but are not limited to, e.g., TQSFNDFTR (SEQ ID NO: 1) and SVSQTSKLTR (SEQ ID NO: 3). Non-limiting exemplary thrombin cleavage sites include, e.g., DFLAEGGGVR (SEQ ID NO: 4), TTKIKPR (SEQ ID NO: 5), LVPRG (SEQ ID NO: 6), DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88), or IEPRSFS (SEQ ID NO: 194), and a sequence comprising, consisting essentially of, or consisting of ALRPR (SEQ ID NO: 7) (e.g., ALRPRVVGGA (SEQ ID NO: 145)).

[0204] In a specific embodiment, the cleavage site is TLDPRSFLLRNPNDKYEPFWEDEEK (SEQ ID NO: 146). In another embodiment, the cleavage site comprises DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88) or a fragment thereof. In one particular embodiment, the cleavage site comprises IEPRSFS (SEQ ID NO: 194). In another embodiment, the cleavage site comprises EPRSFS (SEQ ID NO: 195), wherein the cleavage site is not the full-length a2 region of FVIII. In still another embodiment, the cleavage site comprises IEPR (SEQ ID NO: 200). In another embodiment, the cleavage site comprises IEPR (SEQ ID NO: 200), wherein the cleavage site is not the full-length a2 region of FVIII or does not comprise the full-length a2 region of FVIII. In other embodiments, the cleavage site comprises DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88), KNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 139), NTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 140), TGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 141), GDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 142), DYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 143), YYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 144), YEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 176), EDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 177), DSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 178), SYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 179), YEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 180), EDISAYLLSKNNAIEPRSFS (SEQ ID NO: 181), DISAYLLSKNNAIEPRSFS (SEQ ID NO: 182), ISAYLLSKNNAIEPRSFS (SEQ ID NO: 183), SAYLLSKNNAIEPRSFS (SEQ ID NO: 184), AYLLSKNNAIEPRSFS (SEQ ID NO: 185), YLLSKNNAIEPRSFS (SEQ ID NO: 186), LLSKNNAIEPRSFS (SEQ ID NO: 187), LSKNNAIEPRSFS (SEQ ID NO: 188), SKNNAIEPRSFS (SEQ ID NO: 189), KNNAIEPRSFS (SEQ ID NO: 190), NNAIEPRSFS (SEQ ID NO: 191), NAIEPRSFS (SEQ ID NO: 192), AIEPRSFS (SEQ ID NO: 193), or IEPRSFS (SEQ ID NO: 194). In other embodiments, the cleavage site comprises DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88), KNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 139), NTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 140), TGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 141), GDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 142), DYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 143), YYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 144), YEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 176), EDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 177), DSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 178), SYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 179), YEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 180), EDISAYLLSKNNAIEPRSFS (SEQ ID NO: 181), DISAYLLSKNNAIEPRSFS (SEQ ID NO: 182), ISAYLLSKNNAIEPRSFS (SEQ ID NO: 183), SAYLLSKNNAIEPRSFS (SEQ ID NO: 184), AYLLSKNNAIEPRSFS (SEQ ID NO: 185), YLLSKNNAIEPRSFS (SEQ ID NO: 186), LLSKNNAIEPRSFS (SEQ ID NO: 187), LSKNNAIEPRSFS (SEQ ID NO: 188), SKNNAIEPRSFS (SEQ ID NO: 189), KNNAIEPRSFS (SEQ ID NO: 190), NNAIEPRSFS (SEQ ID NO: 191), NAIEPRSFS (SEQ ID NO: 192), AIEPRSFS (SEQ ID NO: 193), or IEPRSFS (SEQ ID NO: 194), wherein the cleavage site is not the full-length FVIII a2 region. In certain embodiments the cleavable linker is cleavable in a thrombin cleavage assay as provided herein or as known in the art.

III. Polynucleotides, Vectors, and Host cells

[0205] Also provided in the invention is a polynucleotide encoding a chimeric protein of the invention. In one embodiment, the first polypeptide chain and the second polypeptide chain can be encoded by a single

polynucleotide chain. In another embodiment, the first polypeptide chain and the second polypeptide chain are encoded by two different polynucleotides, *i.e.,* a first nucleotide sequence and a second nucleotide sequence. In another embodiment, the first nucleotide sequence and the second nucleotide sequence are on two different polynucleotides (e.g., different vectors).

[0206] Disclosed herein is a polynucleotide encoding a single polypeptide chain (e.g., FVIII(X2)-F1-L3-F2-L2-X1-L1-V), wherein FVIII(X2) comprises a FVIII protein in which an XTEN sequence is inserted at one or more insertion sites, F1 comprises a first Ig constant region or a portion thereof, *e.g.,* a first Fc region, L1 comprises a first linker, V comprises a VWF protein, X1 comprises an XTEN sequence having less than 288 amino acids in length, L2 comprises a second linker, L3 comprises a third linker, and F2 comprises a second Ig constant region or a portion thereof, *e.g.,* a second Fc region. Disclosed herein are also two polynucleotides, a first polynucleotide sequence encoding a first polypeptide which comprises a FVIII protein fused to a first Ig constant region or a portion thereof and a second polynucleotide sequence encoding a second polypeptide which comprises a VWF protein, an XTEN sequence having less than 288 amino acids in length, and a second Ig constant region or a portion thereof. A chimeric protein comprising two polypeptide chains or three polypeptide chains can be encoded by a single polynucleotide chain, and then processed into two or three (or more) polypeptide chains. Also, a chimeric protein comprising these polypeptide chains can be encoded by two or three polynucleotide chains.

[0207] In some embodiments, the set of the polynucleotides further comprises an additional nucleotide chain *(e.g.,* a second nucleotide chain when the chimeric polypeptide is encoded by a single polynucleotide chain or a third nucleotide chain when the chimeric protein is encoded by two polynucleotide chains) which encodes a protein convertase. The protein convertase can be selected from the group consisting of proprotein convertase subtilisin/kexin type 5 (PCSK5 or PC5), proprotein convertase subtilisin/kexin type 7 (PCSK7 or PC5), a yeast Kex 2, proprotein convertase subtilisin/kexin type 3 (PACE or PCSK3), and two or more combinations thereof. In some embodiments, the protein convertase is PACE, PC5, or PC7. In a specific embodiment, the protein convertase is PC5 or PC7. See International Application no. PCT/US2011/043568.

[0208] As used herein, an expression vector refers to any nucleic acid construct which contains the necessary elements for the transcription and translation of an inserted coding sequence, or in the case of an RNA viral vector, the necessary elements for replication and translation, when introduced into an appropriate host cell. Expression vectors can include plasmids, phagemids, viruses, and derivatives thereof.

[0209] Expression vectors of the disclosure will include polynucleotides encoding the chimeric protein described herein. One or more of the coding sequences for the first polypeptide comprising a FVIII protein and a first Ig constant region, the second polypeptide comprising a VWF protein, an XTEN sequence having less than 288 amino acids, and a second Ig constant region or a portion thereof, or both may be operably linked to an expression control sequence. As used herein, two nucleic acid sequences are operably linked when they are covalently linked in such a way as to permit each component nucleic acid sequence to retain its functionality. A coding sequence and a gene expression control sequence are said to be operably linked when they are covalently linked in such a way as to place the expression or transcription and/or translation of the coding sequence under the influence or control of the gene expression control sequence. Two DNA sequences are said to be operably linked if induction of a promoter in the 5' gene expression sequence results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the coding sequence, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a gene expression sequence would be operably linked to a coding nucleic acid sequence if the gene expression sequence were capable of effecting transcription of that coding nucleic acid sequence such that the resulting transcript is translated into the desired protein or polypeptide.

[0210] A gene expression control sequence as used herein is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation

of the coding nucleic acid to which it is operably linked. The gene expression control sequence may, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Constitutive mammalian promoters include, but are not limited to, the promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPRT), adenosine deaminase, pyruvate kinase, beta-actin promoter, and other constitutive promoters. Exemplary viral promoters which function constitutively in eukaryotic cells include, for example, promoters from the cytomegalovirus (CMV), simian virus (e.g., SV40), papilloma virus, adenovirus, human immunodeficiency virus (HIV), Rous sarcoma virus, cytomegalovirus, the long terminal repeats (LTR) of Moloney leukemia virus, and other retroviruses, and the thymidine kinase promoter of herpes simplex virus. Other constitutive promoters are known to those of ordinary skill in the art. The promoters useful as gene expression sequences of the invention also include inducible promoters. Inducible promoters are expressed in the presence of an inducing agent. For example, the metallothionein promoter is induced to promote transcription and translation in the presence of certain metal ions. Other inducible promoters are known to those of ordinary skill in the art.

[0211] In general, the gene expression control sequence shall include, as necessary, 5' non-transcribing and 5' non-translating sequences involved with the initiation of transcription and translation, respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined coding nucleic acid. The gene expression sequences optionally include enhancer sequences or upstream activator sequences as desired.

[0212] Viral vectors include, but are not limited to, nucleic acid sequences from the following viruses: retrovirus, such as Moloney murine leukemia virus, Harvey murine sarcoma virus, murine mammary tumor virus, and Rous sarcoma virus; adenovirus, adeno-associated virus; SV40-type viruses; polyomaviruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors well-known in the art. Certain viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses, the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. Most useful are those retroviruses that are replication-deficient *(i.e.,* capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high efficiency transduction of genes *in vivo.* Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell line with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in Kriegler, M., Gene Transfer and Expression, A Laboratory Manual, W.H. Freeman Co., New York (1990) and Murry, E. J., Methods in Molecular Biology, Vol. 7, Humana Press, Inc., Cliffton, N.J. (1991).

[0213] In one embodiment, the virus is an adeno-associated virus, a double-stranded DNA virus. The adeno-associated virus can be engineered to be replication-deficient and is capable of infecting a wide range of cell types and species. It further has advantages such as heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages, including hematopoietic cells; and lack of superinfection inhibition thus allowing multiple series of transductions. Reportedly, the adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression characteristic of retroviral infection. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion.

[0214] Other vectors include plasmid vectors. Plasmid vectors have been extensively described in the art and are well-known to those of skill in the art. See, *e.g.,* Sambrook et al., Molecular Cloning: A Laboratory Manual,

Second Edition, Cold Spring Harbor Laboratory Press, 1989. In the last few years, plasmid vectors have been found to be particularly advantageous for delivering genes to cells *in vivo* because of their inability to replicate within and integrate into a host genome. These plasmids, however, having a promoter compatible with the host cell, can express a peptide from a gene operably encoded within the plasmid. Some commonly used plasmids available from commercial suppliers include pBR322, pUC18, pUC19, various pcDNA plasmids, pRC/CMV, various pCMV plasmids, pSV40, and pBlueScript. Additional examples of specific plasmids include pcDNA3.1, catalog number V79020; pcDNA3.1/hygro, catalog number V87020; pcDNA4/myc-His, catalog number V86320; and pBudCE4.1, catalog number V53220, all from Invitrogen (Carlsbad, CA.). Other plasmids are well-known to those of ordinary skill in the art. Additionally, plasmids may be custom designed using standard molecular biology techniques to remove and/or add specific fragments of DNA.

[0215] In one insect expression system that may be used to produce the proteins of the invention, *Autographa californica* nuclear polyhidrosis virus (AcNPV) is used as a vector to express the foreign genes. The virus grows in *Spodoptera frugiperda* cells. A coding sequence may be cloned into non-essential regions (for example, the polyhedron gene) of the virus and placed under control of an ACNPV promoter (for example, the polyhedron promoter). Successful insertion of a coding sequence will result in inactivation of the polyhedron gene and production of non-occluded recombinant virus (*i.e.*, virus lacking the proteinaceous coat coded for by the polyhedron gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (see, *e.g.,* Smith et al. (1983) J Virol 46:584; U.S. Pat. No. 4,215,051). Further examples of this expression system may be found in Ausubel et al., eds. (1989) Current Protocols in Molecular Biology, Vol. 2, Greene Publish. Assoc. & Wiley Interscience.

[0216] Another system which can be used to express the proteins of the invention is the glutamine synthetase gene expression system, also referred to as the "GS expression system" (Lonza Biologics PLC, Berkshire UK). This expression system is described in detail in U.S. Pat. No. 5,981,216.

[0217] In mammalian host cells, a number of viral based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, a coding sequence may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing peptide in infected hosts. See, *e.g.,* Logan & Shenk (1984) Proc Natl Acad Sci USA 81:3655). Alternatively, the vaccinia 7.5 K promoter may be used. See, *e.g.,* Mackett et al. (1982) Proc Natl Acad Sci USA 79:7415; Mackett et al. (1984) J Virol 49:857; Panicali et al. (1982) Proc Natl Acad Sci USA 79:4927.

[0218] To increase efficiency of production, the polynucleotides can be designed to encode multiple units of the protein of the invention separated by enzymatic cleavage sites. The resulting polypeptide can be cleaved *(e.g.,* by treatment with the appropriate enzyme) in order to recover the polypeptide units. This can increase the yield of polypeptides driven by a single promoter. When used in appropriate viral expression systems, the translation of each polypeptide encoded by the mRNA is directed internally in the transcript; *e.g.,* by an internal ribosome entry site, IRES. Thus, the polycistronic construct directs the transcription of a single, large polycistronic mRNA which, in turn, directs the translation of multiple, individual polypeptides. This approach eliminates the production and enzymatic processing of polyproteins and may significantly increase the yield of polypeptides driven by a single promoter.

[0219] Vectors used in transformation will usually contain a selectable marker used to identify transformants. In bacterial systems, this can include an antibiotic resistance gene such as ampicillin or kanamycin. Selectable markers for use in cultured mammalian cells include genes that confer resistance to drugs, such as neomycin, hygromycin, and methotrexate. The selectable marker may be an amplifiable selectable marker. One amplifiable selectable marker is the dihydrofolate reductase (DHFR) gene. Simonsen C C et al. (1983) Proc Natl Acad Sci USA 80:2495-9. Selectable markers are reviewed by Thilly (1986) Mammalian Cell Technology, Butterworth Publishers, Stoneham, Mass., and the choice of selectable markers is well within the level of ordinary skill in the

art.

[0220] Selectable markers may be introduced into the cell on a separate plasmid at the same time as the gene of interest, or they may be introduced on the same plasmid. If on the same plasmid, the selectable marker and the gene of interest may be under the control of different promoters or the same promoter, the latter arrangement producing a dicistronic message. Constructs of this type are known in the art (for example, U.S. Pat. No. 4,713,339).

[0221] The expression vectors can encode for tags that permit easy purification of the recombinantly produced protein. Examples include, but are not limited to, vector pUR278 (Ruther et al. (1983) EMBO J 2:1791), in which coding sequences for the protein to be expressed may be ligated into the vector in frame with the lac z coding region so that a tagged fusion protein is produced; pGEX vectors may be used to express proteins of the invention with a glutathione S-transferase (GST) tag. These proteins are usually soluble and can easily be purified from cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The vectors include cleavage sites (thrombin or Factor Xa protease or PRESCISSION PROTEASE™ (Pharmacia, Peapack, N.J.)) for easy removal of the tag after purification.

[0222] The expression vector or vectors are then transfected or co-transfected into a suitable target cell, which will express the polypeptides. Transfection techniques known in the art include, but are not limited to, calcium phosphate precipitation (Wigler et al. (1978) Cell 14:725), electroporation (Neumann et al. (1982) EMBO J 1:841), and liposome-based reagents. A variety of host-expression vector systems may be utilized to express the proteins described herein including both prokaryotic and eukaryotic cells. These include, but are not limited to, microorganisms such as bacteria (*e.g., E. coli*) transformed with recombinant bacteriophage DNA or plasmid DNA expression vectors containing an appropriate coding sequence; yeast or filamentous fungi transformed with recombinant yeast or fungi expression vectors containing an appropriate coding sequence; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing an appropriate coding sequence; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus or tobacco mosaic virus) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing an appropriate coding sequence; or animal cell systems, including mammalian cells (*e.g.,* HEK 293, CHO, Cos, HeLa, HKB11, and BHK cells).

[0223] In one embodiment, the host cell is a eukaryotic cell. As used herein, a eukaryotic cell refers to any animal or plant cell having a definitive nucleus. Eukaryotic cells of animals include cells of vertebrates, *e.g.,* mammals, and cells of invertebrates, *e.g.,* insects. Eukaryotic cells of plants specifically can include, without limitation, yeast cells. A eukaryotic cell is distinct from a prokaryotic cell, e.g., bacteria.

[0224] In certain embodiments, the eukaryotic cell is a mammalian cell. A mammalian cell is any cell derived from a mammal. Mammalian cells specifically include, but are not limited to, mammalian cell lines. In one embodiment, the mammalian cell is a human cell. In another embodiment, the mammalian cell is a HEK 293 cell, which is a human embryonic kidney cell line. HEK 293 cells are available as CRL-1533 from American Type Culture Collection, Manassas, VA, and as 293-H cells, Catalog No. 11631-017 or 293-F cells, Catalog No. 11625-019 from Invitrogen (Carlsbad, Calif.). In some embodiments, the mammalian cell is a PER.C6® cell, which is a human cell line derived from retina. PER.C6® cells are available from Crucell (Leiden, The Netherlands). In other embodiments, the mammalian cell is a Chinese hamster ovary (CHO) cell. CHO cells are available from American Type Culture Collection, Manassas, VA. *(e.g.,* CHO-K1; CCL-61). In still other embodiments, the mammalian cell is a baby hamster kidney (BHK) cell. BHK cells are available from American Type Culture Collection, Manassas, Va. *(e.g.,* CRL-1632). In some embodiments, the mammalian cell is a HKB11 cell, which is a hybrid cell line of a HEK293 cell and a human B cell line. Mei et al., Mol. Biotechnol. 34(2): 165-78 (2006).

[0225] A plasmid including a FVIII(X2)-Fc fusion coding sequence, a VWF protein-L1-X1-L2-Fc coding sequence, or both and a selectable marker, e.g., zeocin resistance, may be transfected into HEK 293 cells, for production of a chimeric protein.

[0226] A plasmid including a FVIII-Fc fusion coding sequence, a VWF protein-L1-X-L2-Fc coding sequence, or both and a selectable marker, e.g., zeocin resistance, may be transfected into HEK 293 cells, for production of a chimeric protein.

[0227] A first plasmid including a FVIII(X2)-Fc fusion coding sequence and a first selectable marker, e.g., a zeocin resistance gene, and a second plasmid including a VWF protein-L1-X1-L2-Fc coding sequence and a second selectable marker, e.g., a neomycin resistance gene, and a third plasmid including a protein convertase coding sequence and a third selectable marker, e.g., a hygromycin resistance gene, may be cotransfected into HEK 293 cells, for production of the chimeric protein. The first and second plasmids can be introduced in equal amounts (*i.e.*, 1:1 molar ratio), or they can be introduced in unequal amounts.

[0228] A first plasmid including a FVIII-Fc fusion coding sequence and a first selectable marker, e.g., a zeocin resistance gene, and a second plasmid including a VWF protein-L1-X-L2-Fc coding sequence and a second selectable marker, e.g., a neomycin resistance gene, and a third plasmid including a protein convertase coding sequence and a third selectable marker, e.g., a hygromycin resistance gene, may be cotransfected into HEK 293 cells, for production of the chimeric protein. The first and second plasmids can be introduced in equal amounts *(i.e.,* 1:1 molar ratio), or they can be introduced in unequal amounts.

[0229] A first plasmid including a FVIII(X2)-Fc fusion coding sequence and a first selectable marker, e.g., a zeocin resistance gene, and a second plasmid including a VWF protein-L1-X1-L2-Fc fusion coding sequence and a second selectable marker, e.g., a neomycin resistance gene, and a third plasmid including a protein convertase coding sequence and a third selectable marker, e.g., a hygromycin resistance gene, may be cotransfected into HEK 293 cells, for production of the chimeric protein. The first and second plasmids can be introduced in equal amounts *(i.e.,* 1:1 molar ratio), or they can be introduced in unequal amounts.

[0230] A first plasmid, including a chimeric protein encoding FVIII (with or without XTEN)-F1-L3-F2-L2-X-L1-V coding sequence and a first selectable marker, e.g., a zeocin resistance gene, and a second plasmid including a protein convertase coding sequence and a second selectable marker, e.g., a hygromycin resistance gene, may be cotransfected into HEK 293 cells, for production of the chimeric protein. The promoters for the FVIII(X)-F1 coding sequence and the V-L2-X-L1-F2 coding sequence can be different or they can be the same.

[0231] In some embodiments, transfected cells are stably transfected. These cells can be selected and maintained as a stable cell line, using conventional techniques known to those of skill in the art.

[0232] Host cells containing DNA constructs of the protein are grown in an appropriate growth medium. As used herein, the term "appropriate growth medium" means a medium containing nutrients required for the growth of cells. Nutrients required for cell growth may include a carbon source, a nitrogen source, essential amino acids, vitamins, minerals, and growth factors. Optionally, the media can contain one or more selection factors. Optionally the media can contain bovine calf serum or fetal calf serum (FCS). In one embodiment, the media contains substantially no IgG. The growth medium will generally select for cells containing the DNA construct by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker on the DNA construct or co-transfected with the DNA construct. Cultured mammalian cells are generally grown in commercially available serum-containing or serum-free media (e.g., MEM, DMEM, DMEM/F12). In one embodiment, the medium is CD293 (Invitrogen, Carlsbad, CA.). In another embodiment, the medium is CD17 (Invitrogen, Carlsbad, CA.). Selection of a medium appropriate for the particular cell line used is within the level of those ordinary skilled in the art.

[0233] In order to co-express the two polypeptide chains of the chimeric protein, the host cells are cultured under conditions that allow expression of both chains. As used herein, culturing refers to maintaining living cells *in vitro* for at least a definite time. Maintaining can, but need not include, an increase in population of living cells. For example, cells maintained in culture can be static in population, but still viable and capable of producing a

desired product, e.g., a recombinant protein or recombinant fusion protein. Suitable conditions for culturing eukaryotic cells are well known in the art and include appropriate selection of culture media, media supplements, temperature, pH, oxygen saturation, and the like. For commercial purposes, culturing can include the use of any of various types of scale-up systems including shaker flasks, roller bottles, hollow fiber bioreactors, stirred-tank bioreactors, airlift bioreactors, Wave bioreactors, and others.

[0234] The cell culture conditions are also selected to allow association of the VWF fragment with the FVIII protein. Conditions that allow expression of the VWF fragment and/or the FVIII protein may include the presence of a source of vitamin K. For example, in one embodiment, stably transfected HEK 293 cells are cultured in CD293 media (Invitrogen, Carlsbad, CA) or OptiCHO media (Invitrogen, Carlsbad, CA) supplemented with 4 mM glutamine.

[0235] In one aspect, the present invention is directed to a method of expressing, making, or producing the chimeric protein of the invention comprising a) transfecting a host cell comprising a polynucleotide encoding the chimeric protein and b) culturing the host cell in a culture medium under a condition suitable for expressing the chimeric protein, wherein the chimeric protein is expressed.

[0236] The protein product containing the FVIII protein linked to a first Ig constant region or a portion thereof and/or the VWF protein fused to a second Ig constant region or a portion thereof by an XTEN sequence may be secreted into the media. Media is separated from the cells, concentrated, filtered, and then passed over two or three affinity columns, *e.g.,* a protein A column and one or two anion exchange columns.

[0237] In certain aspects, the present invention relates to the chimeric protein produced by the methods described herein.

[0238] *In vitro* production allows scale-up to give large amounts of the desired altered polypeptides of the invention. Techniques for mammalian cell cultivation under tissue culture conditions are known in the art and include homogeneous suspension culture, e.g. in an airlift reactor or in a continuous stirrer reactor, or immobilized or entrapped cell culture, e.g. in hollow fibers, microcapsules, on agarose microbeads or ceramic cartridges. If necessary and/or desired, the solutions of polypeptides can be purified by the customary chromatography methods, for example gel filtration, ion-exchange chromatography, hydrophobic interaction chromatography (HIC, chromatography over DEAE-cellulose or affinity chromatography.

IV. Pharmaceutical Composition

[0239] Compositions containing the chimeric protein of the present invention may contain a suitable pharmaceutically acceptable carrier. For example, they may contain excipients and/or auxiliaries that facilitate processing of the active compounds into preparations designed for delivery to the site of action.

[0240] The pharmaceutical composition can be formulated for parenteral administration (*i.e.* intravenous, subcutaneous, or intramuscular) by bolus injection. Formulations for injection can be presented in unit dosage form, *e.g.,* in ampoules or in multidose containers with an added preservative. The compositions can take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, e.g., pyrogen free water.

[0241] Suitable formulations for parenteral administration also include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances, which increase the viscosity of the

suspension, including, for example, sodium carboxymethyl cellulose, sorbitol and dextran. Optionally, the suspension may also contain stabilizers. Liposomes also can be used to encapsulate the molecules of the invention for delivery into cells or interstitial spaces. Exemplary pharmaceutically acceptable carriers are physiologically compatible solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like. In some embodiments, the composition comprises isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride. In other embodiments, the compositions comprise pharmaceutically acceptable substances such as wetting agents or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the active ingredients.

[0242] Compositions of the invention may be in a variety of forms, including, for example, liquid *(e.g.,* injectable and infusible solutions), dispersions, suspensions, semi-solid and solid dosage forms. The preferred form depends on the mode of administration and therapeutic application.

[0243] The composition can be formulated as a solution, micro emulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active ingredient in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active ingredient into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0244] The active ingredient can be formulated with a controlled-release formulation or device. Examples of such formulations and devices include implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, for example, ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for the preparation of such formulations and devices are known in the art. See e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[0245] Injectable depot formulations can be made by forming microencapsulated matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the polymer employed, the rate of drug release can be controlled. Other exemplary biodegradable polymers are polyorthoesters and polyanhydrides. Depot injectable formulations also can be prepared by entrapping the drug in liposomes or microemulsions.

[0246] Supplementary active compounds can be incorporated into the compositions. In one embodiment, the chimeric protein of the invention is formulated with another clotting factor, or a variant, fragment, analogue, or derivative thereof. For example, the clotting factor includes, but is not limited to, factor V, factor VII, factor VIII, factor IX, factor X, factor XI, factor XII, factor XIII, prothrombin, fibrinogen, von Willebrand factor or recombinant soluble tissue factor (rsTF) or activated forms of any of the preceding. The clotting factor of hemostatic agent can also include anti-fibrinolytic drugs, e.g., epsilon-amino-caproic acid, tranexamic acid.

[0247] Dosage regimens may be adjusted to provide the optimum desired response. For example, a single bolus may be administered, several divided doses may be administered over time, or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity

of dosage. See, e.g., Remington's Pharmaceutical Sciences (Mack Pub. Co., Easton, Pa. 1980).

[0248] In addition to the active compound, the liquid dosage form may contain inert ingredients such as water, ethyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils, glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols, and fatty acid esters of sorbitan.

[0249] Non-limiting examples of suitable pharmaceutical carriers are also described in Remington's Pharmaceutical Sciences by E. W. Martin. Some examples of excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, and the like. The composition can also contain pH buffering reagents, and wetting or emulsifying agents.

[0250] For oral administration, the pharmaceutical composition can take the form of tablets or capsules prepared by conventional means. The composition can also be prepared as a liquid for example a syrup or a suspension. The liquid can include suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats), emulsifying agents (lecithin or acacia), non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils), and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations can also include flavoring, coloring and sweetening agents. Alternatively, the composition can be presented as a dry product for constitution with water or another suitable vehicle.

[0251] For buccal administration, the composition may take the form of tablets or lozenges according to conventional protocols.

[0252] For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of a nebulized aerosol with or without excipients or in the form of an aerosol spray from a pressurized pack or nebulizer, with optionally a propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoromethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.,* gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0253] The pharmaceutical composition can also be formulated for rectal administration as a suppository or retention enema, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

[0254] The pharmaceutical composition of the invention comprises a chimeric protein of the invention and a pharmaceutically acceptable carrier. The FVIII protein in a chimeric protein has extended half-life compared to wild type FVIII protein or the corresponding FVIII protein without the VWF fragment. In one embodiment, wherein the half-life of the chimeric protein is extended at least about 1.5 times, at least about 2 times, at least about 2.5 times, at least about 3 times, at least about 4 times, at least about 5 times, at least about 6 times, at least about 7 times, at least about 8 times, at least about 9 times, at least about 10 times, at least about 11 times, or at least about 12 times longer than wild type FVIII. In another embodiment, the half-life of Factor VIII is at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 25 hours, at least about 26 hours, at least about 27 hours, at least about 28 hours, at least about 29 hours, at least about 30 hours, at least about 31 hours, at least about 32 hours, at least about 33 hours, at least about 34 hours, at least about 35 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours.

[0255] In some embodiments, the composition is administered by a route selected from the group consisting of topical administration, intraocular administration, parenteral administration, intrathecal administration, subdural administration and oral administration. The parenteral administration can be intravenous or subcutaneous

administration.

[0256] In other embodiments, the composition is used to treat a bleeding disease or condition in a subject in need thereof. The bleeding disease or condition is selected from the group consisting of a bleeding coagulation disorder, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, bleeding in the illiopsoas sheath and any combinations thereof. In still other embodiments, the subject is scheduled to undergo a surgery. In yet other embodiments, the treatment is prophylactic or on-demand.

V. Gene Therapy

[0257] A chimeric protein thereof of the invention can be produced in vivo in a mammal, e.g., a human patient, using a gene therapy approach to treatment of a bleeding disease or disorder selected from the group consisting of a bleeding coagulation disorder, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, and bleeding in the illiopsoas sheath would be therapeutically beneficial. The bleeding disease or disorder may be hemophilia, e.g. hemophilia A. This involves administration of a suitable chimeric protein-encoding nucleic acid operably linked to suitable expression control sequences. These sequences may be incorporated into a viral vector. Suitable viral vectors for such gene therapy include adenoviral vectors, lentiviral vectors, baculoviral vectors, Epstein Barr viral vectors, papovaviral vectors, vaccinia viral vectors, herpes simplex viral vectors, and adeno associated virus (AAV) vectors. The viral vector can be a replication-defective viral vector. An adenoviral vector may have a deletion in its E1 gene or E3 gene. When an adenoviral vector is used, the mammal may not be exposed to a nucleic acid encoding a selectable marker gene. The sequences may be incorporated into a non-viral vector known to those skilled in the art.

V. Methods of Using Chimeric Protein

[0258] The present invention is directed to a chimeric protein of the invention for use in a method to prevent or inhibit endogenous VWF binding to a FVIII protein. Disclosed is a method of using a chimeric protein having a FVIII protein linked to XTEN and an Ig constant region or a portion thereof.

[0259] One aspect is directed to preventing or inhibiting FVIII interaction with endogenous VWF by blocking or shielding the VWF binding site on the FVIII from endogenous VWF and at the same time extending half-life of the chimeric protein using an XTEN sequence in combination with an Ig constant region or a portion thereof, which can also be a half-life extender. Disclosed is a method of constructing a FVIII protein having half-life longer than wild-type FVIII. The chimeric protein useful in the method includes any one or more chimeric protein described herein.

[0260] Also disclosed is a method of administering to a subject in need thereof a chimeric protein comprising a FVIII protein having half-life longer than wild-type FVIII, wherein the method comprises administering the chimeric protein described herein to the subject.

[0261] Also disclosed is a method of using an XTEN sequence and an Ig constant region or a portion thereof to improve a half-life of a chimeric protein comprising FVIII protein and a VWF protein, which prevents or inhibits endogenous VWF interaction with a FVIII protein. A FVIII protein linked to an XTEN sequence (e.g., FVIII(X)) and then bound to or associated with a VWF protein fused to an XTEN and an Ig constant region or a portion thereof

is shielded or protected from the clearance pathway of VWF and thus has reduced clearance compared to the FVIII protein not bound to the VWF protein. The shielded FVIII protein thus has maximum extension of a half-life compared to a FVIII protein not bound to or associated with the XTEN sequence and the VWF protein. In one aspect, the FVIII protein associated with or protected by a VWF protein and linked to an XTEN sequence is not cleared by a VWF clearance receptor. The FVIII protein associated with or protected by a VWF protein and linked to an XTEN sequence may be cleared from the system slower than the FVIII protein that is not associated with or protected by the VWF protein and linked to the XTEN sequence.

[0262] In one aspect, the chimeric protein comprising the FVIII protein linked to an XTEN sequence or the FVIII protein bound to or associated with a VWF protein linked to XTEN has reduced clearance from circulation as the VWF protein does not contain a VWF clearance receptor binding site. The VWF protein prevents or inhibits clearance of FVIII bound to or associated with the VWF protein from the system through the VWF clearance pathway. The VWF proteins can also provide at least one or more VWF-like FVIII protection properties that are provided by endogenous VWF. The VWF protein or the XTEN sequence can also mask one or more FVIII clearance receptor binding site, thereby preventing clearance of FVIII by its own clearance pathway.

[0263] The prevention or inhibition of a FVIII protein binding to endogenous VWF by the VWF protein or the XTEN sequence can be *in vitro* or in vivo.

[0264] Also disclosed is a method of increasing the half-life of a chimeric protein comprising administering the chimeric protein described herein to a subject in need thereof. The half-life of non-activated FVIII bound to or associated with full-length VWF is about 12 to 14 hours in plasma. In VWD type 3, wherein there is almost no VWF in circulation, the half-life of FVIII is only about six hours, leading to symptoms of mild to moderate hemophilia A in such patients due to decreased concentrations of FVIII. The half-life of the chimeric protein linked to or associated with the VWF fragment or the XTEN sequence of the present invention can increase at least about 1.5 times, 1.6 times, 1.7 times, 1.8 times, 1.9 times, 2.0 times, 2.1 times, 2.2 times, 2.3 times, 2.4 times, 2.6 times, 2.7. times, 2.8 times, 2.9 times, 3.0 times, 3.1 times, 3.2 times, 3.3 times, 3.4 times, 3.5 times, 3.6 times, 3.7 times, 3.8 times, 3.9 times, or 4.0 times higher than the half-life of the non-activated FVIII bound to or associated with full-length VWF.

[0265] A chimeric protein comprising a first polypeptide comprising a FVIII protein and a first Ig constant region or a portion thereof and a second polypeptide comprising a VWF protein, an XTEN having less than 288 amino acids, and an Ig constant region or a portion thereof may exhibit a half-life at least about 2 times, 2.5 times, 3.0 times, 3.5 times, 4.0 times, 4.5 times, 5.0 times, 5.5 times, 6.0 times, 7 times, 8 times, 9 times, or 10 times higher than a corresponding chimeric protein comprising the same first polypeptide and the second polypeptide without the XTEN sequence or wild type FVIII. A chimeric protein comprising a first polypeptide comprising a FVIII protein and a first Ig constant region or a portion thereof and a second polypeptide comprising a VWF protein, an XTEN having less than 288 amino acids, and an Ig constant region or a portion thereof may exhibit a half-life about 2 to about 5 times, about 3 to about 10 times, about 5 to about 15 times, about 10 to about 20 times, about 15 to about 25 times, about 20 to about 30 times, about 25 to about 35 times, about 30 to about 40 times, about 35 to about 45 times higher than a corresponding chimeric protein comprising the same first polypeptide and the second polypeptide without the XTEN sequence or wild type FVIII. In a specific embodiment, the half-life of a chimeric protein of the invention increases at least about 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 times higher than the half-life of the wild type FVIII in a FVIII and VWF double knockout mouse.

[0266] In certain embodiments, a chimeric protein exhibits a half-life of about 40 hours in mice.

[0267] In some embodiments, the half-life of a chimeric protein is longer than the half-life of a FVIII associated with endogenous VWF. In other embodiments, the half-life of the chimeric protein is at least about 1.5 times, 2 times, 2.5 times, 3.5 times, 3.6 times, 3.7 times, 3.8 times, 3.9 times, 4.0 times, 4.5 times, or 5.0 times the half-life of wild type FVIII or a FVIII protein associated with endogenous VWF.

[0268] In some embodiments, as a result of the invention the half-life of the chimeric protein is extended compared to a FVIII protein without the VWF fragment or wild-type FVIII. The half-life of the chimeric protein of the invention is at least about 1.5 times, at least about 2 times, at least about 2.5 times, at least about 3 times, at least about 4 times, at least about 5 times, at least about 6 times, at least about 7 times, at least about 8 times, at least about 9 times, at least about 10 times, at least about 11 times, or at least about 12 times longer than the half-life of a chimeric protein without the VWF fragment or wild-type FVIII. In one embodiment, the half-life of FVIII is about 1.5-fold to about 20-fold, about 1.5 fold to about 15 fold, or about 1.5 fold to about 10 fold longer than the half-life of wild-type FVIII. In another embodiment, the half-life of the FVIII is extended about 2-fold to about 10-fold, about 2-fold to about 9-fold, about 2-fold to about 8-fold, about 2-fold to about 7-fold, about 2-fold to about 6-fold, about 2-fold to about 5-fold, about 2-fold to about 4-fold, about 2-fold to about 3-fold, about 2.5-fold to about 10-fold, about 2.5-fold to about 9-fold, about 2.5-fold to about 8-fold, about 2.5-fold to about 7-fold, about 2.5-fold to about 6-fold, about 2.5-fold to about 5-fold, about 2.5-fold to about 4-fold, about 2.5-fold to about 3-fold, about 3-fold to about 10-fold, about 3-fold to about 9-fold, about 3-fold to about 8-fold, about 3-fold to about 7-fold, about 3-fold to about 6-fold, about 3-fold to about 5-fold, about 3-fold to about 4-fold, about 4-fold to about 6 fold, about 5-fold to about 7-fold, or about 6-fold to about 8 fold as compared to wild-type FVIII or a FVIII protein without the VWF fragment. In other embodiments, the half-life of the chimeric protein of the invention is at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 25 hours, at least about 26 hours, at least about 27 hours, at least about 28 hours, at least about 29 hours, at least about 30 hours, at least about 31 hours, at least about 32 hours, at least about 33 hours, at least about 34 hours, at least about 35 hours, at least about 36 hours, at least about 40 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours. In still other embodiments, the half-life of the chimeric protein of the invention is about 15 hours to about two weeks, about 16 hours to about one week, about 17 hours to about one week, about 18 hours to about one week, about 19 hours to about one week, about 20 hours to about one week, about 21 hours to about one week, about 22 hours to about one week, about 23 hours to about one week, about 24 hours to about one week, about 36 hours to about one week, about 48 hours to about one week, about 60 hours to about one week, about 24 hours to about six days, about 24 hours to about five days, about 24 hours to about four days, about 24 hours to about three days, or about 24 hours to about two days.

[0269] In some embodiments, the average half-life of the chimeric protein of the invention per subject is about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours (1 day), about 25 hours, about 26 hours, about 27 hours, about 28 hours, about 29 hours, about 30 hours, about 31 hours, about 32 hours, about 33 hours, about 34 hours, about 35 hours, about 36 hours, about 40 hours, about 44 hours, about 48 hours (2 days), about 54 hours, about 60 hours, about 72 hours (3 days), about 84 hours, about 96 hours (4 days), about 108 hours, about 120 hours (5 days), about six days, about seven days (one week), about eight days, about nine days, about 10 days, about 11 days, about 12 days, about 13 days, or about 14 days.

[0270] In addition, the invention provides a chimeric protein for use in a method of treating or preventing a bleeding disease or disorder comprising administering an effective amount of a chimeric protein. In one embodiment, the bleeding disease or disorder is selected from the group consisting of a bleeding coagulation disorder, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, and bleeding in the iliopsoas sheath. In a specific embodiment, the bleeding disease or disorder is hemophilia A.

[0271] The chimeric protein comprising an XTEN sequence and an Ig constant region or a portion thereof in combination with a VWF protein described herein, that prevents or inhibits interaction of the FVIII protein with endogenous VWF prepared according to the present disclosure, has many uses as will be recognized by one skilled in the art, including, but not limited to methods of treating a subject having a hemostatic disorder and

methods of treating a subject in need of a general hemostatic agent. In one embodiment, the invention relates to the chimeric protein of the invention for use in a method of treating a subject having a hemostatic disorder comprising administering a therapeutically effective amount of the chimeric protein.

[0272] The FVIII protein portion in the chimeric protein treats or prevents a hemostatic disorder by serving as a cofactor to Factor IX on a negatively charged phospholipid surface, thereby forming a Xase complex. The binding of activated coagulation factors to a phospholipid surface localizes this process to sites of vascular damage. On a phospholipid surface, Factor VIIIa increases the maximum velocity of Factor X activation by Factor IXa, by approximately 200,000-fold, leading to the large second burst of thrombin generation.

[0273] The chimeric protein of the invention can be used to treat any hemostatic disorder. The hemostatic disorders that may be treated by administration of the chimeric protein of the invention include, but are not limited to, hemophilia A, as well as deficiencies or structural abnormalities relating to Factor VIII. In one embodiment, the hemostatic disorder is hemophilia A.

[0274] The chimeric protein of the invention can be used prophylactically to treat a subject with a hemostatic disorder. The chimeric protein of the invention can be used to treat an acute bleeding episode in a subject with a hemostatic disorder. In another embodiment, the hemostatic disorder can be the result of a defective clotting factor, e.g., von Willebrand's factor. In one embodiment, the hemostatic disorder is an inherited disorder. In another embodiment, the hemostatic disorder is an acquired disorder. The acquired disorder can result from an underlying secondary disease or condition. The unrelated condition can be, as an example, but not as a limitation, cancer, an auto-immune disease, or pregnancy. The acquired disorder can result from old age or from medication to treat an underlying secondary disorder (*e.g.* cancer chemotherapy).

[0275] The invention also relates to the chimeric protein of the invention for use in methods of treating a subject that does not have a congenital hemostatic disorder, but has a secondary disease or condition resulting in acquisition of a hemostatic disorder, e.g., due to development of an anti-FVIII antibody or a surgery. The invention thus relates to the chimeric protein of the invention for use in a method of treating a subject in need of a general hemostatic agent comprising administering a therapeutically effective amount of the chimeric protein prepared by the present methods.

[0276] The present invention is also related to the chimeric protein of the invention for use in methods of reducing immunogenicity of FVIII or inducing less immunogenicity against FVIII comprising administering an effective amount of the chimeric protein or the polynucleotides encoding the same.

[0277] In one embodiment, the subject in need of a general hemostatic agent is undergoing, or is about to undergo, surgery. The chimeric protein of the invention can be administered prior to, during, or after surgery as a prophylactic regimen. The chimeric protein of the invention can be administered prior to, during, or after surgery to control an acute bleeding episode.

[0278] The chimeric protein of the invention can be used to treat a subject having an acute bleeding episode who does not have a hemostatic disorder. The acute bleeding episode can result from severe trauma, e.g., surgery, an automobile accident, wound, laceration gun shot, or any other traumatic event resulting in uncontrolled bleeding. Non limiting examples of bleeding episodes include a bleeding coagulation disorder, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, bleeding in the iliopsoas sheath, and any combinations thereof.

[0279] In prophylactic applications, one or more compositions containing the chimeric protein of the invention or a cocktail thereof are administered to a patient not already in the disease state to enhance the patient's resistance or reduce symptoms associated with a disease or disorder. Such an amount is defined to be a

"prophylactic effective dose." In therapeutic applications, a relatively high dosage (e.g., from about 1 to 400 mg/kg of polypeptide per dose, with dosages of from 5 to 25 mg being more commonly used for radioimmunoconjugates and higher doses for cytotoxin-drug modified polypeptides) at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

[0280] In some embodiments, a chimeric protein or a composition of the invention is used for on-demand treatment, which includes treatment for a bleeding episode, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis (head trauma), gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, or bleeding in the iliopsoas sheath. The subject may be in need of surgical prophylaxis, peri-operative management, or treatment for surgery. Such surgeries include, e.g., minor surgery, major surgery, tooth extraction, tonsillectomy, inguinal herniotomy, synovectomy, total knee replacement, craniotomy, osteosynthesis, trauma surgery, intracranial surgery, intra-abdominal surgery, intrathoracic surgery, or joint replacement surgery.

[0281] In one embodiment, the chimeric protein of the present invention is administered intravenously, subcutaneously, intramuscularly, or via any mucosal surface, e.g., orally, sublingually, buccally, nasally, rectally, vaginally or via pulmonary route. The chimeric protein comprising a VWF fragment and a FVIII protein of the present invention can be implanted within or linked to a biopolymer solid support that allows for the slow release of the chimeric protein to the site of bleeding or implanted into bandage/dressing. The dose of the chimeric protein will vary depending on the subject and upon the particular route of administration used. Dosages can range from 0.1 to 100,000 µg/kg body weight. In one embodiment, the dosing range is 0.1-1,000 µg/kg. In another embodiment, the dosing range is 0.1-500 µg/kg. The protein can be administered continuously or at specific timed intervals. *In vitro* assays may be employed to determine optimal dose ranges and/or schedules for administration. *In vitro* assays that measure clotting factor activity are known in the art, e.g., STA-CLOT VIIa-rTF clotting assay or ROTEM clotting assay. Additionally, effective doses may be extrapolated from dose-response curves obtained from animal models, *e.g.,* a hemophiliac dog (Mount et al. 2002, Blood 99(8):2670).

[0282] Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for purposes of illustration only and are not intended to be limiting of the invention.

Examples

[0283] Throughout the examples, the following materials and methods were used unless otherwise stated.

Materials and Methods

[0284] In general, the practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, biophysics, molecular biology, recombinant DNA technology, immunology (especially, *e.g.,* antibody technology), and standard techniques in electrophoresis. See, *e.g.,* Sambrook, Fritsch and Maniatis, Molecular Cloning: Cold Spring Harbor Laboratory Press (1989); Antibody Engineering Protocols (Methods in Molecular Biology), 510, Paul, S., Humana Pr (1996); Antibody Engineering: A Practical Approach (Practical Approach Series, 169), McCafferty, Ed., Irl Pr (1996); Antibodies: A Laboratory Manual, Harlow et al., CS.H.L. Press, Pub. (1999); and Current Protocols in Molecular Biology, eds. Ausubel et al., John Wiley & Sons (1992).

Example 1: FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers

[0285] The present invention is directed to generate a chimeric FVIII molecule which is coupled to D'D3 domain of von Willebrand Factor (VWF) protein via Fc domain of IgG. Attached D'D3 domain prevents the interaction of FVIII with endogenous VWF multimers. This molecule serves as a platform to incorporate other half- life extension technologies in order to improve the pharmacokinetics of the chimeric protein. XTEN sequences were incorporated into the FVIII B-domain and in between D'D3 and Fc region to increase the half-life of FVIII/VWF heterodimer.

[0286] Thrombin cleavage site in between D'D3 and Fc allows the release of D'D3 domain upon the activation of FVIII molecule by thrombin.

Example 2: Plasmid construction of FVIII-XTEN-Fc/D'D3-Fc heterodimers

Cloning of VWF050- IHH triple mutation in VWF031

[0287] IHH triple mutation in Fc prevents interaction with FcRn, thus there is no recycling of Fc containing molecule by FcRn pathway. The 3 mutations in Fc are I253A, H310A, H435A.

[0288] VWF050 was generated by swapping the Fc region of VWF031 plasmid with Fc fragment containing IHH triple mutation between the RsRII and Not 1 restriction sites.

[0289] Cloning of VWF057- Cloning VWF-Fc with 144 AE XTEN +35aa thrombin cleavable linker.

Oligos

[0290]

ESC 155- Oligo for 144 AE XTEN in VWF034-rev
CCCCGCCACCGGATCCCCCGCCACCGGATCCCCCGCCACCGGATCCCCCGCCACCGGAACCTC
CACCGCCGCTCGAGGCACCTTCTTCAGTGCTGGTGGGCGAGCCCGCTGGTGACCCTTCCTC

ESC 156-Oligo for 144 AE XTEN- GS linker in VWF034-rev
GGGGAAGAGGAAGACTGACGGTCCGCCCAGGAGTTCTGGAGCTGGGCACGGTGGGCATGTGT
GAGTTTTGTCGCCTCCGCTGCCCCGGGGGACCAGGGATCCCCCGCCACCGGATCCCCCGCCAC
CGGATCCCCCGCCACCGGATCCCCCGCC

ESC 157-Oligo for 144 AE XTEN in VWF031-Fwd
GTGAAGCCTGCCAGGAGCCGATATCGGGCGCGCCAACATCAGAGAGCGCCACCCCTGAAAGT
GGTCCCGGGAGCGAGCCAGC

[0291] PCR was done twice to obtain the 144 AE-XTEN + 35 aa GS linker with thrombin cleavage site.

[0292] First PCR reaction was done using 144- AE XTEN coding DNA as template and ESC 157/ESC155 primer pair. About 550 bp long PCR product obtained from this reaction was used as template for second PCR reaction and was amplified using ESC 157/156 primer pair. This reaction gave ~ 700 bp long product. This 700 bp PCR product and VWF034 plasmid was then digested with EcoRV-HF and RsRII. Plasmid backbone from digested.

[0293] VWF034 was then used to ligate 700bp PCR product.

Cloning of VWF058- IHH triple mutation in VWF034

[0294] IHH triple mutation in Fc prevents interaction with FcRn, thus there is no recycling of Fc containing molecule by FcRn pathway. The 3 mutations in Fc are I253A, H310A, H435A.

[0295] VWF058 was generated by swapping the Fc region of VWF034 plasmid with Fc fragment containing IHH triple mutation between the RsRII and Not 1 restriction sites.

Cloning of FVIII-263- FVIII 205 with IHH triple mutation

[0296] IHH triple mutation in Fc prevents interaction with FcRn, thus there is no recycling of Fc containing molecule by FcRn pathway. The 3 mutations in Fc are I253A, H310A, H435A.

[0297] FVIII-263 was generated by swapping the Fc region of FVIII 205 plasmid with Fc fragment containing IHH triple mutation between the RsRII and Not 1 restriction sites.

Cloning of FVIII-282- FVIII-Fc with 144 AE XTEN in B-domain

[0298]

ESC 158-Oligo for 144 AE XTEN in B-domain-fwd
AAGAAGCTTCTCTCAAAACGGCGCGCCAACATCAGAGAGCGCCACCCCTGAAAGTGGTCCCG
GGAGCGAGCCAGCCACATCTGGGTCGGAAACGCCAGGC

ESC 159-Oligo for 144 AE XTEN in B-domain-rev
GGTATCATCATAATCGATTTCCTCTTGATCTGACTGAAGAGTAGTACGAGTTATTTCAGCTTGA
TGGCGTTTCAAGACTGGTGGGCTCGAGGCACCTTCTTCAGTGCTGGTGGGCGAGCCCGCTGGT
GACCCTTCCTCAGTGGACGTAGG

[0299] First PCR reaction was done using 144- AE XTEN coding DNA as template and ESC 158/ESC159 primer pair. About 550 bp long PCR product obtained from this reaction and FVIII 169 plasmid was then digested with AscI and Cla1. Plasmid backbone from digested FVIII 169 was then used to ligate 550 bp PCR product in order to obtain FVIII 282.

Cloning of FVIII-283- FVIII 169 with IHH triple mutation

[0300] IHH triple mutation in Fc prevents interaction with FcRn, thus there is no recycling of Fc containing molecule by FcRn pathway. The 3 mutations in Fc are I253A, H310A, H435A.

[0301] FVIII-283 was generated by swapping the Fc region of FVIII 169 plasmid with Fc fragment containing IHH triple mutation between the RsRII and Not 1 restriction sites.

Example 3: Production of FVIII-XTEN-Fc/D'D3-XTEN-Fc in HEK293 cells

[0302] Figure 2. Schematic diagram showing the expression of FVIII-XTEN-Fc/D'D3-XTEN-Fc construct. Three plasmids co-transfection was done in HEK293 cells using Polyethylenimine (PEI). First plasmid derives the

expression of FVIII-XTEN-Fc, second plasmid expresses D1D2DD3-XTEN-Fc and the third plasmid expression PACE/furin, which is required to enzymatically remove propeptide, i.e., D1D2 domain from D1D2D'D3-XTEN-Fc. Products of this three plasmid expression system includes of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimer, D'D3-XTEN-Fc homodimer and traces of FVIII-XTEN-Fc hemizygous looking species.

Example 4: Purification of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers

[0303] To purify the FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers, a tangential flow filtration (TFF) step was used to first concentrate the conditioned media by 10 fold. Products in the filtrate were then further purified using affinity chromatography follow by a desalting column. Purity of the molecule was acceptable by HPLC-SEC and was further confirmed by western blotting. The specific activity of the molecule was comparable to B-domain deleted FVIII, as measured by FVIII activity assay (example 5) and OD280 measurement.

Example 5: Specific activity of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers

[0304] The activity of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers was measure by FVIII chromogenic assay and activated Partial Thromboplastin Time (aPTT) assay. The specific chromogenic activity and specific aPTT activity of SQ BDD-FVIII, rFVIII169/VWF034 and rFVIII169/VWF057 were listed in Table 15. Compared to SQ BDD-FVIII, we have observed comparable specific chromogenic activities and 60% reduction on the specific aPTT activity for rFVIII169/VWF034 and rFVIII169/VWF057.

Table 15: Specific activity of heterodimer variants

FVIII	SQ BDD-FVIII	rFVIII169/VWF034	rFVIII160/VWF057
Specific Chromogenic Activity (IU/pmol)	0.9-2.0	1.1-1.2	0.8-1.6
Specific aPTT Activity (IU/pmol)	0.75-1.7	0.4	0.3-0.6

FVIII chromogenic assay

[0305] The FVIII activity was measured using the COATEST SP FVIII kit from DiaPharma (produce #: K824086) and all incubations were performed on a 37°C plate heater with shaking.

[0306] The WHO 8th International Standard for Blood Coagulation Factor VIII:C, Concentrate, coded 07/350 was used as assay standard, the range of the standard was from 100 mIU/mL to 0.78 mIU/mL. A pooled normal human plasma assay control and testing samples (diluted with IX Coatest buffer) were added into Immulon 2HB 96-well plates in duplicate (25 µL/well). Freshly prepared IXa/FX/Phospholipid mix (50 µL), 25 µL of 25mM $CaCl_2$, and 50 µL of FXa substrate were added sequentially into each well with 5 minutes incubation between each addition. After incubating with the substrate, 25 µL of 20% Acetic Acid was added to terminate the color reaction, and the absorbance of OD405 was measured with a SpectraMAX plus (Molecular Devices) instrument. Data were analyzed with SoftMax Pro software (version 5.2). The Lowest Level of Quantification (LLOQ) is 7.8 mIU/mL.

FVIII aPTT assay

[0307] The FVIII aPTT assay was performed on the Sysmex CA-1500 coagulation analyzer as follows: First, 50 uL of manually diluted samples, standards and Controls in aPTT buffer (50 mM Tris, 100 mM NaCl, 1% HSA, pH 7.4) were added by the instrument into the reaction cuvette, followed by adding 50 uL of FVIII-deficient plasma

(George King Bio-Medical, product #: 0800). Following incubation at 37°C for 1 minute, 50 uL of aPTT reagent (Actin® FSL activated cephaloplastin reagent - Dade Behring, reference # B4219-2) was added to the reaction mixture, and incubated at 37° C for 4 minutes. Subsequently, 50 ul of 20 mM $CaCl_2$ (Dade Behring, reference # ORFO37) was added, and the reaction cuvette was immediately transferred to one of four spectrophotometer channel positions to measure the amount of refracted light in the mixture, which was converted to the onset of clotting by the instrument's software algorithm. Reported clotting time was the length of time from the addition of $CaCl_2$ until the onset of clot formation. Assay standard was generated by diluting the WHO 8th International FVIII Standard into aPTT buffer in a range from 100 mIU/ml to 0.78 mIU/ml. The standard curve was plotted as the clotting time (in seconds) as Y-axis versus the log (base 10) of the FVIII activity (mIU/mL) as X-axis in MS Excel, and the activity of the individual samples was calculated using the formula for the linear regression line of this standard curve. Based on the assay performance, the lower limit of quantization (LLOQ) was 7.8 mIU/mL.

Example 6: Additive effect of XTEN insertions on the half-life extension of heterodimer

[0308] XTEN insertions were incorporated into the heterodimers for half-life extension. Insertion of a single 288 amino acid (aa) AE-XTEN at FVIII B-domain resulted in a 16.7 hrs half-life of the heterodimer in HemA mice, as demonstrated by rFVIII169/VWF031 in Figure 3. To further improve the half-life of the heterodimer, a second XTEN insertion at 144 aa or 288 aa length was incorporated into FVIII169/VWF031 either in the FVIII A1 domain or immediately down stream of D'D3 fragment respectively, the heterodimer variants were named as FVIII205/VWF031 and FVIII169/VWF034.

[0309] The half-life of rFVIII169/VWF031, rFVIII205/VWF031 and rFVIII169/VWF034 were evaluated in FVIII deficient (HemA) mice by a single intravenous administration of test molecules at 200 IU/kg dose. Plasma samples were collected at designate time points as indicated in Figure 3, the FVIII activity of the samples were determined by FVIII chromogenic assay, the PK parameters were calculated using WinNonlin-Phoenix program and listed in Table 16.

[0310] As shown in Figure 3 and Table 16, the addition of the second XTEN insertion either at A1 domain of FVIII or down stream of D'D3 further improves the half-life of heterodimer to 29.45 or 31.10 respectively. Furthermore, more than 2-fold improvements on clearance and AUC were also observed from both XTEN insertions.

Table 16: PK parameter of heterodimers in HemA mice

FVIII	XTEN Insertions		$T_{1/2}$ (hr)	MRT (hr)	Cl (mL/hr/kg)	Vss (mL/kg)	AUC_D (kg*hr/mL)
	Insertion 1	Insertion 2					
rFVIII169/VWF031	B*-AE288		16.65	18.44	3.57	85.72	0.28
rFVIII205/VWF031	B*-AE288	A1-144XTEN	29.45	36.02	1.76	63.56	0.57
rFVIII169/VWF034	B*-AE288	D'D3-AE288	31.10	34.57	1.73	59.77	0.58

Example 7: 144 aa AE-XTEN confers better half-life benefit then 288 aa AE-XTEN when inserted in between D'D3 and Fc domains.

[0311] Another heterodimer-FVIII169/VWF057 was constructed in the effort of identifying the optimal length of XTEN insertion within the D'D3-XTEN-Fc chain, in which the length of XTEN insertion was reduced to 144aa from 288aa. As shown in Figure 4, compared to rFVIII169/VWF034, the half-life of rFVIII169/VWF057 was increased from 31 hrs to 42 hrs. Improved clearance and AUC were also observed for rFVIII169/VWF057, data

was listed in Table 17. Thus, 144aa AE-XTEN insertion is more optimal than AE-288aa XTEN when inserted between D'D3 and Fc domain of the FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers.

Table 17: PK parameters of rFVIII169/VWF034 and rFVIII169/VWF057 in HemA mice

FVIII	$T_{1/2}$ (hr)	MRT (hr)	Cl (mL/hr/kg)	Vss (mL/kg)	AUC_D (kg*hr/mL)
rFVIII169/VWF034	31.10	34.57	1.73	59.77	0.58
rFVIII169/VWF057	42.23	53.24	0.97	51.44	1.03

Example 8: Fc domain extents the half-life of heterodimer

[0312] Fc domains extent its fusion protein's half-life through FcRn mediated recycling pathway. To confirm the necessity of the Fc domain on the half-life extension of the heterodimer, the wild-type Fc domains were replaced by a triple mutant (I253A/H310A/H435A; IHH) in rFVIII205/VWF031 to form rFVIII263/VWF050, and complete elimination of FcRn binding was confirmed by Surface Plasmon Resonance (Biacore) assay for rFVIII263/VWF050. The half-life of FVIII263/VWF050 was evaluated in HemA mice in comparison with rFVIII205/VWF031. Increased clearance rate, as well as reduced half-life and AUC were observed for rFVIII263/VWF050 as shown in Figure 5 and Table 18. This result demonstrated that in addition to ensure the covalent binding of FVIII and D'D3, the Fc domains is also necessary for the half-life improvement of the heterodimer.

Table 18: PK parameters of rFVIII205/VWF031 and rFVIII263/VWF040 in HemA mice

FVIII	Mutation in Fc domain	$T_{1/2}$ (hr)	MRT (hr)	Cl (mL/hr/kg)	Vss (mL/kg)	AUC_D (kg*hr/mL)
rFVIII205/VWF031	None	29.45	36.02	1.76	63.56	0.57
rFVIII263/VWF050	IHH	22.96	26.15	2.36	61.69	0.42

Example 9: Acute efficacy of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers in HemA mouse tail clip bleeding model

[0313] The acute efficacy of lead heterodimer candidates were evaluated using HemA mouse tail clip bleeding model.

[0314] 8-12 weeks old male HemA mice were randomized into 4 treatment groups, and treated with a single intravenous administration of SQ BDD-FVIII, rFVIII169/VWF034, rFVIII169/VWF057 or vehicle solution respectively. In order to mimic the episodic treatment of FVIII (to reconstitute 50-100% of normal FVIII plasma level), the selected FVIII treatment dose is 75 IU/kg as measured by FVIII aPTT activity. At this dose level, all testing FVIII variants will reconstitute ~70% of normal murine plasma FVIII activity 5min post dosing.

[0315] Blood loss volume from each individual animal in the study was plotted in Figure 6. Significant reduction on blood loss volume was observed for all FVIII treatment groups compared to vehicle treated animals. Within the three FVIII treatment groups, no statistical significant different were found on blood loss reduction, suggesting the heterodimer molecules could potentially as efficacious as SQ BDD-FVIII for on demand treatment.

[0316] Blood loss volume from each individual animal in the study was plotted in Figure 6. Significant reduction on blood loss volume was observed for all FVIII treatment groups compared to vehicle treated animals. Within the three FVIII treatment groups, no statistical significant different were found on blood loss reduction, suggesting the heterodimer molecules could potentially as efficacious as SQ BDD-FVIII for on demand

treatment.

[0317] In addition, HemA mice were treated with a lower dose (37.5 IU/kg) of rBDD-FVIII or rFVIII169/VWF034, and the results are shown in Figure 6B. Same as the 75 IU/kg dose, rFVIII169/VWF034 provided similar protection as BDD-FVIII to HemA mice post tail clip injury, indicating the molecule was still efficacious to treat severe bleeding episodes at ~35% of normal murine circulating FVIII level in HemA mice.

[0318] The Tail Clip procedure was carried out as follows. Briefly, mice were anesthetized with a 50 mg/kg Ketamine/0.5 mg/kg Dexmedetomidine cocktail prior to tail injury and placed on a 37°C heating pad to help maintain the body temperature. The tails of the mice were then be immersed in 37°C saline for 10 minutes to dilate the lateral vein. After vein dilation, FVIII variants or vehicle solution were injected via the tail vein and the distal 5 mm of the tail was then cut off using a straight edged #11 scalpel 5min post dosing. The shed blood was collected into 13 ml of 37°C saline for 30 minutes and blood loss volume was determined by the weight change of the blood collection tube: blood loss volume = (collection tube end weight - beginning weight + 0.10) ml. Statistical analysis were conducted using t test (Mann Whitney test) and one way ANOVA (KRUSKAL-Wallis test, posttest: Dunns multiple comparison test).

Example 10: Prophylactic efficacy of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimer in HemA mouse tail vein transection bleeding model

[0319] The prophylactic efficacy of FVIII169/VWF057 was tested in HemA mouse tail vein transection (TVT) model. The TVT model induces bleeding by introducing injury to the lateral vein of the mouse tail, which mimics the spontaneous bleeding episodes in patients with hemophilia bleeding disorder.

[0320] 8-10 weeks old male HemA mice were randomized into four treatment groups, and treated with either FVIII169/VWF057 at 72hr prior of the tail vein injury, or SQ BDD-FVIII at 24hr or 48hr before the injury. Vehicle treated animal were used as negative control. Events of re-bleeding or euthanasia due to the excessive blood loss within 24 hrs post injury were plotted in Figure 7.

[0321] As shown in Figure 7, unlike mice treated with SQ BDD-FVIII at 48hr prior to TVT, of whom only limited protection was observed post injury, mice that received rFVIII169/VWF057 at 72hr prior the tail injury had similar protection on re-bleeding and survival compared to the mice that received SQ BDD-FVIII treatment 24hr before TVT, indicating rFVIII169/VWF057 can provide at least 3-fold or more (e.g., 4-fold) longer-protection to HemA mice in TVT model. Therefor rFVIII169/VWF057 might significantly reduce the treatment frequency of the current FVIII prophylaxis.

[0322] Similarly, HemA mice were treated with FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers: rFVIII169/VWF034 and rFVIII169/VWF057.at 24 or 96 hours prior to the tail vein injury. The rebleeding and survival data of the treatments were compared with the data by the rBDD-FVIII at 24 or 48 hour prior to the injury and vehicle. While the rebleeding in mice treated with rBDD-FVIII at 24 hours prior to the tail vein injury was similar to the mice treated with vehicle, the rebleeding data of mice treated with the heterodimers at 24hr before the injury are significantly better than the vehicle treatment group. Furthermore, the rebleeding data of mice treated with the heterodimers at 96hr before the injury were comparable to mice received rBDD-FVIII at 24hr before the injury. As for the survival rate at 24hr post the TVT injury, in contrast of the less than 50% survival rate of mice treated with rBDD-FVIII, more than 90% of the mice survived the TVT injury with FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers treatment when FVIII molecules were administered at 24hr before the injury. In addition, the survival in mice treated with the FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers at 96 hours prior to the tail vein injury were better (in the case of rFVIII169/VWF034) or comparable (in the case of rFVIII169/VWF057) when compared with the mice that received rBDD-FVIII treatment at 24 hours prior to the injury. Both rebleeding and survival data had indicated a 4-fold efficacy prolongation of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimer treatment vs. rBDD-FVIII treatment.

HemA mouse tail vein transection model

[0323] The tail vein transection procedure was conducted as follows. Mice were anesthetized with a cocktail containing 50 mg/kg of Ketamine, 0.125 mg/kg of Dexmedetomidine, and 0.1 mg/kg of Buprenex. At an adequate anesthetic depth, the lateral tail vein of the mice was transected with straight edged number 11 surgical blade at an area where the diameter of the tail is approximately 2.7 mm. The shedding blood was washed away with warm saline to ensure clear observation of the wound. The treated mice were then single housed in a clean cage with white paper bedding for the next 24 hours. Tail re-bleed and the mouse's physical activity were observed and recorded hourly up to 12 hour post tail injury. Moribund mice were euthanized immediately, and a final observation was performed at 24 hour post tail injury. To mimic the bleeding situation in hemophilia patients and to ensure the animal's completely recovery from anesthesia, 1 mg/kg of Atipamezole solution was given to reverse Dexmedetomidine effect at the beginning of the Tail Vein Transection. An additional dose of 0.1 mg/kg Buprenex was administered at the end of the 12 hour observation period for overnight pain management. The survival curve of Time to Re-bleed and Time to Euthanasia was generated for data analysis, and Log-rank (Mantel-COX) test was used for statistic evaluation.

Example 11: Preparation of FVIII169/VWF059 and other constructs

pSYN FVIII 310 cloning:

[0324] A synthetic DNA fragment flanked with BamHI site at the N-terminus and Cla 1 site at the C-terminus was commercially made. This synthetic DNA was used to replace the BamHI to Cla1 region in pSYN FVIII 169 construct (SEQ ID NO: 155). Both synthetic DNA and pSYN FVIII 169 DNA were double digested with BamHI and Cla1, digested synthetic DNA was inserted into digested pSYN FVIII 169 to create pSYN FVIII 310 (SEQ ID NO:168; Table 19).

[0325] Cloning pSYN FVIII 312:
A synthetic DNA fragment flanked with BamHI site at the N-terminus and Afe 1 site at the C-terminus was commercially made. This synthetic DNA was used to replace the BamHI to Afe1 region in pSYN FVIII 169 construct (SEQ ID NO: 155). Both synthetic DNA and pSYN FVIII 169 DNA were double digested with BamHI and Afe1, digested synthetic DNA was inserted into digested pSYN FVIII 169 to create pSYN FVIII 312 (SEQ ID NO: 169; Table 19). pSYN FVIII 312A (SEQ ID NO: 2; Table 19) was created from pSYN FVIII312 to remove AscI site which codes for amino acid residues GAP at the junction of FVIII and XTEN.

Table 19: Synthetic FVIII constructs.

Construct	Protein Sequence
pSYN FVIII 169	PRSFSQNGAPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPASSPPVLKRHQAEITR (SEQ ID NO: 167)
	(Underlined = XTEN residues; not underlined = FVIII residues)
pSYN FVIII 310	PRSFGAPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPASSEITR (SEQ ID NO: 168)

Construct	Protein Sequence
	[illegible]
	(Underlined = XTEN residues; not underlined = FVIII residues)
pSYN FVIII 312	PRSFSQNGAPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPASSEITR (SEQ ID NO: 169)
	(Underlined = XTEN residues; not underlined = FVIII residues)
pSYN FVIII 312A	PRSFSQNGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPASSEITR (SEQ ID NO: 2)
	(similar sequence as pSYN FVIII312 just residues corresponding to AscI site i.e GAP are removed) (Underlined = XTEN residues; not underlined = FVIII residues)

Cloning pSYN VWF059 and VWF073:

[0326] Various synthetic DNA fragments coding for different linker regions between D'D3-XTEN and Fc were made. These synthetic DNA fragments were flanked with Asc1 site at N-terminus and Not 1 site at the C-terminus. These synthetic DNAs were used to replace the Asc1 to Not1 region in pSYN VWF057 construct (SEQ ID NO: 152). The pSYN VWF059 construct (Table 20) comprises a linker region (SEQ ID NO: 13), which includes the entire FVIII acidic region 2 (a2). This site is reported to be cleaved by thrombin, and upon FVIII activation D'D3XTEN is released. The pSYN VWF073 construct (Table 20) contains only the thrombin cleavage site of FVIII acidic region 2 (a2) (*i.e.,* IEPRSFS) (SEQ ID NO: 23). Both synthetic DNA and pSYN VWF057 DNA were double digested with Asc1 and Not1. Digested synthetic DNA was inserted into digested pSYN VWF057 to create pSYN VWF059 and pSYN VWF073. The pSYN VWF59A construct (Table 20) was generated from pSYN VWF059 by removing the EcoRV restriction site. FVIII169/VWF057 and FVIII169/VWF059 heterodimer proteins were generated by co-expression of FVIII169 and VWF057 or VWF059 in HEK293 cells.

Table 20: Synthetic VWF constructs - Cleavable Linker Regions.

Construct	Protein Sequence
pSYN VWF057	TSTEEGASSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSLVPRGSGGDKTH (SEQ ID NO: 12)
	Italics and underlined sequence shows GS linker and LVPR thrombin cleavage site (also shaded).
pSYN VWF059	TSTEEGASIS*DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS*DKTH (SEQ ID NO: 13)
	Italics and underlined sequence shows 32 aa from FVIII acidic region 2 (a2). Shaded sequence shows thrombin cleavage site used in pSYN VWF059A.
pSYN VWF059A	TSTEEGASS*DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS*DKTH (SEQ ID NO: 22)
	Italics and underlined sequence shows 32 aa from FVIII acidic region 2 (a2). This sequence is similar sequence to VWF059, except that residues corresponding to the EcoRV site (*i.e.,* IS) are removed.
pSYN	TSTEEGASS*GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSIEPRSFSGSGG*DKTH

Construct	Protein Sequence
VWF073	(SEQ ID NO: 23)
	Italics and underlined sequence shows GS linker with truncated thrombin cleavage site from FVIII acidic region 2 (shaded 7 amino acids-IEPRSFS).

Example 12: Thrombin digestion of FVIII heterodimer to analyze the release of D'D3 from Fc

[0327] Two FVIII heterodimer proteins were tested in thrombin digestion experiments and their rate of cleavage by thrombin was examined. The two heterodimer constructs used in this experiment were FVIII169/VWF057 heterodimer and FVIII169/VWF059 heterodimer along with FVIIIFc. The FVIII169/VWF057 and FVIII169/VWF059 heterodimers are described above. Three digestion reactions were carried out: i) FVIIIFc ii) FVIII169/VWF057 (FIG. 11), and iii) FVIII 169/VWF059 (FIG. 12). Test samples were treated with human α-thrombin at a molar ratio if FVIII:thrombin of approximately 22:1. Each reaction was incubated in a 37°C water bath. At each indicated time point (t = 5, 15, 30, 45, 60 minutes), a 22.5 µL sample was withdrawn, stopped with 22.5 µL non-reducing 2x SDS loading dye, and heated for 3 minutes. The digested protein was then run on an SDS-PAGE gel. Western blotting was performed using anti-FVIII heavy chain (GMA012) and anti-VWF-D3 (Ab96340) antibodies using a LICOR system.

[0328] As shown in figure 11, exposure of FVIII169/VWF057 to thrombin resulted in a gradual decrease in the detected level of D'D3-XTEN-Fc, correlating with an increase in the level of D'D3-144 XTEN, the cleaved product. Un-cleaved FVIII169/VWF057 remained after 15 minutes. Conversely, figure 12 shows that FVIII 169/VWF059 is cleaved more rapidly by thrombin, as evidenced by little to no detectable un-cleaved FVIII 169/VWF059 after 5 minutes. Accordingly, FVIII 169/VWF059 showed better release of D'D3 from Fc upon thrombin activation as compared or FVIII169/VWF057.

[0329] Parallel experiments were done to investigate thrombin cleavage using mass spectroscopy (MS). By MS, FVIII 169/VWF059 again showed better release of D'D3 from Fc as compared to VWF057.

Example 13: *In vivo* evaluation of FVIII169/VWF059 in HemA mice

[0330] To further evaluate the pharmacokinetic profile and *in vivo* potency of FVIII169/VWF059, HemA mice were treated with FVIII169/VWF059 through intravenous administration at 150 IU/kg dose. Plasma samples were collected via vena cava blood collection at 5 minutes, 24, 48, 72, 96 and 120 hours post injection. FVIII activity in plasma samples were measured by FVIII chromogenic assay and PK parameters were calculated using Phoenix program. A similar PK profile of FVIII169/VWF059 was observed in comparison with FVIII169/VWF057, as shown in Table 21, indicating that the a2 thrombin cleavage linker has no negative effect on the PK profile of the heterodimer.

Table 21: PK profile of FVIII169/VWF057 and FVIII169/VWF059 in HemA mice

Heterodimer	$T_{1/2}$ (hr)	AUC/D (hr*kg*mIU/mL/mIU)	Cl (mL/hr/kg)	MRT (hr)	Vss (mL/kg)
FVIII169/VWF057	38.53	0.80	1.26	44.92	56.38
FVIII169/VWF059	40.51	0.74	1.35	49.22	66.26

[0331] The acute efficacy of FVIII169/VWF059 was evaluated in a HemA mouse tail clip model (described in Example 9) in comparison with wild type BDD-FVIII. HemA mice were treated with 75 IU/kg of either FVIII169/VWF059 or BDD-FVIII, and blood loss volume of each experimental mouse was plotted in Figure 13.

Compared to BDD-FVIII, FVIII169/VWF059 provided the same degree of protection to HemA mice (p=0.9883), indicating that FVIII169/VWF059 is fully functional *in vivo*.

Plasmid construction of FVIII-XTEN-Fc/D'D3-Fc heterodimers

[0332]

VWF031 nucleotide Sequence (SEQ ID NO: 147)

```
   1    ATGAT TCCTG CCAGA TTTGC CGGGG TGCTG CTTGC TCTGG CCCTC ATTTT
  51    GCCAG GGACC CTTTG TGCAG AAGGA ACTCG CGGCA GGTCA TCCAC GGCCC

 101    GATGC AGCCT TTTCG GAAGT GACTT CGTCA ACACC TTTGA TGGGA GCATG
 151    TACAG CTTTG CGGGA TACTG CAGTT ACCTC CTGGC AGGGG GCTGC CAGAA
 201    ACCCT CCTTC TCCAT TATTC GCCAC TTCCA GAATG CCAAG ACAGT GACCC
 251    TCTCC GTGTA TCTTG GGGAA TTTTT TGACA TCCAT TTGTT TGTCA ATGGT
 301    ACCGT GACAC AGGGG GACCA AAGAG TCTCC ATGCC CTATG CCTCC AAAGG
 351    GCTGT ATCTA GAAAC TGAGG CTGGG TACTA CAAGC TGTCC GGTGA GGCCT
 401    ATGGC TTTGT GGCCA GGATC GATGG CAGCG GCAAC TTTCA AGTCC TGCTG
 451    TCAGA CAGAT ACTTC AACAA GACCT GCGGG CTGTG TGGCA ACTTT AACAT
 501    CTTTG CTGAA GATGA CTTTA TGACC CAAGA AGGGA CCTTG ACCTC GGACC
 551    CTTAT GACTT TGCCA ACTCA TGGGC TCTGA GCAGT GGAGA ACAGT GGTGT
 601    GAACG GGCAT CTCCT CCCAG CAGCT CATGC AACAT CTCCT CTGGG GAAAT
 651    GCAGA AGGGC CTGTG GGAGC AGTGC CAGCT TCTGA AGAGC ACCTC GGTGT
 701    TTGCC CGCTG CCACC CTCTG GTGGA CCCCG AGCCT TTTGT GGCCC TGTGT
 751    GAGAA GACTT TGTGT GAGTG TGCTG GGGGG CTGGA GTGCG CCTGC CCTGC
 801    CCTCC TGGAG TACGC CCGGA CCTGT GCCCA GGAGG GAATG GTGCT GTACG
 851    GCTGG ACCGA CCACA GCGCG TGCAG CCCAG TGTGC CCTGC TGGTA TGGAG
 901    TATAG GCAGT GTGTG TCCCC TTGCG CCAGG ACCTG CCAGA GCCTG CACAT
 951    CAATG AAATG TGTCA GGAGC GATGC GTGGA TGGCT GCAGC TGCCC TGAGG
1001    GACAG CTCCT GGATG AAGGC CTCTG CGTGG AGAGC ACCGA GTGTC CCTGC
1051    GTGCA TTCCG GAAAG CGCTA CCCTC CCGGC ACCTC CCTCT CTCGA GACTG
1101    CAACA CCTGC ATTTG CCGAA ACAGC CAGTG GATCT GCAGC AATGA AGAAT
1151    GTCCA GGGGA GTGCC TTGTC ACTGG TCAAT CCCAC TTCAA GAGCT TTGAC
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1351    CTGCA CAACA GCCTT GTGAA ACTGA AGCAT GGGGC AGGAG TTGCC ATGGA
1401    TGGCC AGGAC ATCCA GCTCC CCCTC CTGAA AGGTG ACCTC CGCAT CCAGC
1451    ATACA GTGAC GGCCT CCGTG CGCCT CAGCT ACGGG GAGGA CCTGC AGATG
1501    GACTG GGATG GCCGC GGGAG GCTGC TGGTG AAGCT GTCCC CCGTC TATGC
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1601    ACTTC CTTAC CCCCT CTGGG CTGGC GGAGC CCCGG GTGGA GGACT TCGGG
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1701    CGATC CCTGC GCCCT CAACC CGCGC ATGAC CAGGT TCTCC GAGGA GGCGT
1751    GCGCG GTCCT GACGT CCCCC ACATT CGAGG CCTGC CATCG TGCCG TCAGC
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1951    AACTG CCCGA AAGGC CAGGT GTACC TGCAG TGCGG GACCC CCTGC AACCT
2001    GACCT GCCGC TCTCT CTCTT ACCCG GATGA GGAAT GCAAT GAGGC CTGCC
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2101    TGCGT GCCCA AGGCC CAGTG CCCCT GTTAC TATGA CGGTG AGATC TTCCA
2151    GCCAG AAGAC ATCTT CTCAG ACCAT CACAC CATGT GCTAC TGTGA GGATG
2201    GCTTC ATGCA CTGTA CCATG AGTGG AGTCC CCGGA AGCTT GCTGC CTGAC
2251    GCTGT CCTCA GCAGT CCCCT GTCTC ATCGC AGCAA AAGGA GCCTA TCCTG
2301    TCGGC CCCCC ATGGT CAAGC TGGTG TGTCC CGCTG ACAAC CTGCG GGCTG
2351    AAGGG CTCGA GTGTA CCAAA ACGTG CCAGA ACTAT GACCT GGAGT GCATG
2401    AGCAT GGGCT GTGTC TCTGG CTGCC TCTGC CCCCC GGGCA TGGTC CGGCA
2451    TGAGA ACAGA TGTGT GGCCC TGGAA AGGTG TCCCT GCTTC CATCA GGGCA
2501    AGGAG TATGC CCCTG GAGAA ACAGT GAAGA TTGGC TGCAA CACTT GTGTC
2551    TGTCG GGACC GGAAG TGGAA CTGCA CAGAC CATGT GTGTG ATGCC ACGTG
2601    CTCCA CGATC GGCAT GGCCC ACTAC CTCAC CTTCG ACGGG CTCAA ATACC
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2751    CTCAG TGAAA TGCAA GAAAC GGGTC ACCAT CCTGG TGGAG GGAGG AGAGA
2801    TTGAG CTGTT TGACG GGGAG GTGAA TGTGA AGAGG CCCAT GAAGG ATGAG
2851    ACTCA CTTTG AGGTG GTGGA GTCTG GCCGG TACAT CATTC TGCTG CTGGG
2901    CAAAG CCCTC TCCGT GGTCT GGGAC CGCCA CCTGA GCATC TCCGT GGTCC
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3051    CCCTG TGGAC TTTGG GAACT CCTGG AAAGT GAGCT CGCAG TGTGC TGACA
3101    CCAGA AAAGT GCCTC TGGAC TCATC CCCTG CCACC TGCCA TAACA ACATC
3151    ATGAA GCAGA CGATG GTGGA TTCCT CCTGT AGAAT CCTTA CCAGT GACGT
3201    CTTCC AGGAC TGCAA CAAGC TGGTG GACCC CGAGC CATAT CTGGA TGTCT
3251    GCATT TACGA CACCT GCTCC TGTGA GTCCA TTGGG GACTG CGCCG CATTC
3301    TGCGA CACCA TTGCT GCCTA TGCCC ACGTG TGTGC CCAGC ATGGC AAGGT
3351    GGTGA CCTGG AGGAC GGCCA CATTG TGCCC CCAGA GCTGC GAGGA GAGGA
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3401    ATCTC CGGGA GAACG GGTAT GAGGC TGAGT GGCGC TATAA CAGCT GTGCA
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3751    GGATC CGGCG GTGGA GGTTC CGGCG GTGGA GGTTC CGGTG GCGGG GGATC
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3851    GTGGC GGGGG ATCCG ACAAA ACTCA CACAT GCCCA CCGTG CCCAG CTCCA
3901    GAACT CCTGG GCGGA CCGTC AGTCT TCCTC TTCCC CCCAA AACCC AAGGA
3951    CACCC TCATG ATCTC CCGGA CCCCT GAGGT CACAT GCGTG GTGGT GGACG
4001    TGAGC CACGA AGACC CTGAG GTCAA GTTCA ACTGG TACGT GGACG GCGTG
4051    GAGGT GCATA ATGCC AAGAC AAAGC CGCGG GAGGA GCAGT ACAAC AGCAC
4101    GTACC GTGTG GTCAG CGTCC TCACC GTCCT GCACC AGGAC TGGCT GAATG
4151    GCAAG GAGTA CAAGT GCAAG GTCTC CAACA AAGCC CTCCC AGCCC CCATC
4201    GAGAA AACCA TCTCC AAAGC CAAAG GGCAG CCCCG AGAAC CACAG GTGTA
4251    CACCC TGCCC CCATC CCGCG ATGAG CTGAC CAAGA ACCAG GTCAG CCTGA
4301    CCTGC CTGGT CAAAG GCTTC TATCC CAGCG ACATC GCCGT GGAGT GGGAG
4351    AGCAA TGGGC AGCCG GAGAA CAACT ACAAG ACCAC GCCTC CCGTG TTGGA
4401    CTCCG ACGGC TCCTT CTTCC TCTAC AGCAA GCTCA CCGTG GACAA GAGCA
4451    GGTGG CAGCA GGGGA ACGTC TTCTC ATGCT CCGTG ATGCA TGAGG CTCTG
4501    CACAA CCACT ACACG CAGAA GAGCC TCTCC CTGTC TCCGG GTAAA TGA
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VWF031 protein Sequence (SEQ ID NO: 86)

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1           MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFVNTFDGSM
      51    YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
     101    TVTQGDQRVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
     151    SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
     201    ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
     251    EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
     301    YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTECPC
     351    VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD
     401    NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG
     451    LHNSLVKLKH GAGVAMDGQD IQLPLLKGDL RIQHTVTASV RLSYGEDLQM
     501    DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
     551    NAWKLHGDCQ DLQKQHSDPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
     601    PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCEL
     651    NCPKGQVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP PGLYMDERGD
     701    CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
     751    AVLSSPLSHR SKRSLSCRPP MVKLVCPADN LRAEGLECTK TCQNYDLECM
     801    SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
     851    CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGECQ YVLVQDYCGS
     901    NPGTFRILVG NKGCSHPSVK CKKRVTILVE GGEIELFDGE VNVKRPMKDE
     951    THFEVVESGR YIILLLGKAL SVVWDRHLSI SVVLKQTYQE KVCGLCGNFD
    1001    GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
    1051    MKQTMVDSSC RILTSDVFQD CNKLVDPEPY LDVCIYDTCS CESIGDCAAF
    1101    CDTIAAYAHV CAQHGKVVTW RTATLCPQSC EERNLRENGY EAEWRYNSCA
    1151    PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
    1201    VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP ISGGGGSGGG

1251    GSGGGGSGGG GSGGGGSGGG GSLVPRGSGG GGSGGGGSDK THTCPPCPAP
1301    ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
1351    EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI
1401    EKTISKAKGQ PREPQVYTLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE
1451    SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL
1501    HNHYTQKSLS LSPGK*
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VWF034 nucleotide Sequence (SEQ ID NO: 148)

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 101    GATGC AGCCT TTTCG GAAGT GACTT CGTCA ACACC TTTGA TGGGA GCATG
 151    TACAG CTTTG CGGGA TACTG CAGTT ACCTC CTGGC AGGGG GCTGC CAGAA
 201    ACGCT CCTTC TCGAT TATTG GGGAC TTCCA GAATG GCAAG AGAGT GAGCC
 251    TCTCC GTGTA TCTTG GGGAA TTTTT TGACA TCCAT TTGTT TGTCA ATGGT
 301    ACCGT GACAC AGGGG GACCA AAGAG TCTCC ATGCC CTATG CCTCC AAAGG
 351    GCTGT ATCTA GAAAC TGAGG CTGGG TACTA CAAGC TGTCC GGTGA GGCCT
 401    ATGGC TTTGT GGCCA GGATC GATGG CAGCG GCAAC TTTCA AGTCC TGCTG
 451    TCAGA CAGAT ACTTC AACAA GACCT GCGGG CTGTG TGGCA ACTTT AACAT
 501    CTTTG CTGAA GATGA CTTTA TGACC CAAGA AGGGA CCTTG ACCTC GGACC
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 601    GAACG GGCAT CTCCT CCCAG CAGCT CATGC AACAT CTCCT CTGGG GAAAT
 651    GCAGA AGGGC CTGTG GGAGC AGTGC CAGCT TCTGA AGAGC ACCTC GGTGT
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 751    GAGAA GACTT TGTGT GAGTG TGCTG GGGGG CTGGA GTGCG CCTGC CCTGC
 801    CCTCC TGGAG TACGC CCGGA CCTGT GCCCA GGAGG GAATG GTGCT GTACG
 851    GCTGG ACCGA CCACA GCGCG TGCAG CCCAG TGTGC CCTGC TGGTA TGGAG
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 951    CAATG AAATG TGTCA GGAGC GATGC GTGGA TGGCT GCAGC TGCCC TGAGG
1001    GACAG CTCCT GGATG AAGGC CTCTG CGTGG AGAGC ACCGA GTGTC CCTGC
1051    GTGCA TTCCG GAAAG CGCTA CCCTC CCGGC ACCTC CCTCT CTCGA GACTG
1101    CAACA CCTGC ATTTG CCGAA ACAGC CAGTG GATCT GCAGC AATGA AGAAT
1151    GTCCA GGGGA GTGCC TTGTC ACTGG TCAAT CCCAC TTCAA GAGCT TTGAC
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1201    AACAG ATACT TCACC TTCAG TGGGA TCTGC CAGTA CCTGC TGGCC CGGGA
1251    TTGCC AGGAC CACTC CTTCT CCATT GTCAT TGAGA CTGTC CAGTG TGCTG
1301    ATGAC CGCGA CGCTG TGTGC ACCCG CTCCG TCACC GTCCG GCTGC CTGGC
1351    CTGCA CAACA GCCTT GTGAA ACTGA AGCAT GGGGC AGGAG TTGCC ATGGA
1401    TGGCC AGGAC ATCCA GCTCC CCCTC CTGAA AGGTG ACCTC CGCAT CCAGC
1451    ATACA GTGAC GGCCT CCGTG CGCCT CAGCT ACGGG GAGGA CCTGC AGATG
1501    GACTG GGATG GCCGC GGGAG GCTGC TGGTG AAGCT GTCCC CCGTC TATGC
1551    CGGGA AGACC TGCGG CCTGT GTGGG AATTA CAATG GCAAC CAGGG CGACG
1601    ACTTC CTTAC CCCCT CTGGG CTGGC GGAGC CCCGG GTGGA GGACT TCGGG
1651    AACGC CTGGA AGCTG CACGG GGACT GCCAG GACCT GCAGA AGCAG CACAG
1701    CGATC CCTGC GCCCT CAACC CGCGC ATGAC CAGGT TCTCC GAGGA GGCGT
1751    GCGCG GTCCT GACGT CCCCC ACATT CGAGG CCTGC CATCG TGCCG TCAGC
1801    CCGCT GCCCT ACCTG CGGAA CTGCC GCTAC GACGT GTGCT CCTGC TCGGA
1851    CGGCC GCGAG TGCCT GTGCG GCGCC CTGGC CAGCT ATGCC GCGGC CTGCG
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2001    GACCT GCCGC TCTCT CTCTT ACCCG GATGA GGAAT GCAAT GAGGC CTGCC
2051    TGGAG GGCTG CTTCT GCCCC CCAGG GCTCT ACATG GATGA GAGGG GGGAC
2101    TGCGT GCCCA AGGCC CAGTG CCCCT GTTAC TATGA CGGTG AGATC TTCCA
2151    GCCAG AAGAC ATCTT CTCAG ACCAT CACAC CATGT GCTAC TGTGA GGATG
2201    GCTTC ATGCA CTGTA CCATG AGTGG AGTCC CCGGA AGCTT GCTGC CTGAC
2251    GCTGT CCTCA GCAGT CCCCT GTCTC ATCGC AGCAA AAGGA GCCTA TCCTG
2301    TCGGC CCCCC ATGGT CAAGC TGGTG TGTCC CGCTG ACAAC CTGCG GGCTG
2351    AAGGG CTCGA GTGTA CCAAA ACGTG CCAGA ACTAT GACCT GGAGT GCATG
2401    AGCAT GGGCT GTGTC TCTGG CTGCC TCTGC CCCCC GGGCA TGGTC CGGCA

2451    TGAGA ACAGA TGTGT GGCCC TGGAA AGGTG TCCCT GCTTC CATCA GGGCA
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2601    CTCCA CGATC GGCAT GGCCC ACTAC CTCAC CTTCG ACGGG CTCAA ATACC
2651    TGTTC CCCGG GGAGT GCCAG TACGT TCTGG TGCAG GATTA CTGCG GCAGT
2701    AACCC TGGGA CCTTT CGGAT CCTAG TGGGG AATAA GGGAT GCAGC CACCC
2751    CTCAG TGAAA TGCAA GAAAC GGGTC ACCAT CCTGG TGGAG GGAGG AGAGA
2801    TTGAG CTGTT TGACG GGGAG GTGAA TGTGA AGAGG CCCAT GAAGG ATGAG
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3001    GGCAT CCAGA ACAAT GACCT CACCA GCAGC AACCT CCAAG TGGAG GAAGA
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3101    CCAGA AAAGT GCCTC TGGAC TCATC CCCTG CCACC TGCCA TAACA ACATC
3151    ATGAA GCAGA CGATG GTGGA TTCCT CCTGT AGAAT CCTTA CCAGT GACGT
3201    CTTCC AGGAC TGCAA CAAGC TGGTG GACCC CGAGC CATAT CTGGA TGTCT
3251    GCATT TACGA CACCT GCTCC TGTGA GTCCA TTGGG GACTG CGCCG CATTC
3301    TGCGA CACCA TTGCT GCCTA TGCCC ACGTG TGTGC CCAGC ATGGC AAGGT
3351    GGTGA CCTGG AGGAC GGCCA CATTG TGCCC CCAGA GCTGC GAGGA GAGGA
3401    ATCTC CGGGA GAACG GGTAT GAGGC TGAGT GGCGC TATAA CAGCT GTGCA
3451    CCTGC CTGTC AAGTC ACGTG TCAGC ACCCT GAGCC ACTGG CCTGC CCTGT
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3551    ATGAG CTTTT GCAGA CCTGC GTTGA CCCTG AAGAC TGTCC AGTGT GTGAG
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3651    TGACC CTGAG CACTG CCAGA TTTGC CACTG TGATG TTGTC AACCT CACCT
3701    GTGAA GCCTG CCAGG AGCCG ATATC GGGTA CCTCA GAGTC TGCTA CCCCC
3751    GAGTC AGGGC CAGGA TCAGA GCCAG CCACC TCCGG GTCTG AGACA CCCGG
3801    GACTT CCGAG AGTGC CACCC CTGAG TCCGG ACCCG GGTCC GAGCC CGCCA
3851    CTTCC GGCTC CGAAA CTCCC GGCAC AAGCG AGAGC GCTAC CCCAG AGTCA
3901    GGACC AGGAA CATCT ACAGA GCCCT CTGAA GGCTC CGCTC CAGGG TCCCC
3951    AGCCG GCAGT CCCAC TAGCA CCGAG GAGGG AACCT CTGAA AGCGC CACAC
4001    CCGAA TCAGG GCCAG GGTCT GAGCC TGCTA CCAGC GGCAG CGAGA CACCA
4051    GGCAC CTCTG AGTCC GCCAC ACCAG AGTCC GGACC CGGAT CTCCC GCTGG
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4201    TCAGA GTCGG CAACC CCGGA GTCTG GACCC GGAAC TTCCG AAAGT GCCAC
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4551    CCCAG GCACT AGTAC CGAGC CCTCT GAAGG CAGTG CGCCA GATTC TGGCG
4601    GTGGA GGTTC CGGTG GCGGG GGATC CGGTG GCGGG GGATC CGGTG GCGGG
4651    GGATC CGGTG GCGGG GGATC CCTGG TCCCC CGGGG CAGCG GAGGC GACAA
4701    AACTC ACACA TGCCC ACCGT GCCCA GCTCC AGAAC TCCTG GGCGG ACCGT
4751    CAGTC TTCCT CTTCC CCCCA AAACC CAAGG ACACC CTCAT GATCT CCCGG
4801    ACCCC TGAGG TCACA TGCGT GGTGG TGGAC GTGAG CCACG AAGAC CCTGA
4851    GGTCA AGTTC AACTG GTACG TGGAC GGCGT GGAGG TGCAT AATGC CAAGA
4901    CAAAG CCGCG GGAGG AGCAG TACAA CAGCA CGTAC CGTGT GGTCA GCGTC
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5101    GATGA GCTGA CCAAG AACCA GGTCA GCCTG ACCTG CCTGG TCAAA GGCTT
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5201    ACAAC TACAA GACCA CGCCT CCCGT GTTGG ACTCC GACGG CTCCT TCTTC
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5301    CTTCT CATGC TCCGT GATGC ATGAG GCTCT GCACA ACCAC TACAC GCAGA
5351    AGAGC CTCTC GCTGT CTCCG GGTAA ATGA
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5351 AGAGC CTCTC CCTGT CTCCG GGTAA ATGA

VWF034 Protein Sequence (SEQ ID NO: 87)

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 251  EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
 301  YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTECPC
 351  VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD
 401  NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG
 451  LHNSLVKLKH GAGVAMDGQD IQLPLLKGDL RIQHTVTASV RLSYGEDLQM
 501  DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
 551  NAWKLHGDCQ DLQKQHSDPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
 601  PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCEL
 651  NCPKGQVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP PGLYMDERGD
 701  CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
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1351  GTSESATPES GPGSPAGSPT STEEGSPAGS PTSTEEGTST EPSEGSAPGT
1401  SESATPESGP GTSESATPES GPGTSESATP ESGPGSEPAT SGSETPGSEP
1451  ATSGSETPGS PAGSPTSTEE GTSTEPSEGS APGTSTEPSE GSAPGSEPAT
1501  SGSETPGTSE SATPESGPGT STEPSEGSAP DIGGGGGSGG GGSLVPRGSG
1551  GDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
1601  DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
1651  KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV
1701  KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
1751  GNVFSCSVMH EALHNHYTQK SLSLSPGK*
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VWF050 nucleotide sequence (IHH triple mutant) (SEQ ID NO: 149)

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 301  ACCGT GACAC AGGGG GACCA AAGAG TCTCC ATGCC CTATG CCTCC AAAGG
 351  GCTGT ATCTA GAAAC TGAGG CTGGG TACTA CAAGC TGTCC GGTGA GGCCT
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1351  CTGCA CAACA GCCTT GTGAA ACTGA AGCAT GGGGC AGGAG TTGCC ATGGA
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1451  ATACA GTGAC GGCCT CCGTG CGCCT CAGCT ACGGG GAGGA CCTGC AGATG
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2151    GCCAG AAGAC ATCTT CTCAG ACCAT CACAC CATGT GCTAC TGTGA GGATG
2201    GCTTC ATGCA CTGTA CCATG AGTGG AGTCC CCGGA AGCTT GCTGC CTGAC
2251    GCTGT CCTCA GCAGT CCCCT GTCTC ATCGC AGCAA AAGGA GCCTA TCCTG
2301    TCGGC CCCCC ATGGT CAAGC TGGTG TGTCC CGCTG ACAAC CTGCG GGCTG
2351    AAGGG CTCGA GTGTA CCAAA ACGTG CCAGA ACTAT GACCT GGAGT GCATG
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2451    TGAGA ACAGA TGTGT GGCCC TGGAA AGGTG TCCCT GCTTC CATCA GGGCA
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2551    TGTCG GGACC GGAAG TGGAA CTGCA CAGAC CATGT GTGTG ATGCC ACGTG
2601    CTCCA CGATC GGCAT GGCCC ACTAC CTCAC CTTCG ACGGG CTCAA ATACC
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2851    ACTCA CTTTG AGGTG GTGGA GTCTG GCCGG TACAT CATTC TGCTG CTGGG
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3001    GGCAT CCAGA ACAAT GACCT CACCA GCAGC AACCT CCAAG TGGAG GAAGA
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3151    ATGAA GCAGA CGATG GTGGA TTCCT CCTGT AGAAT CCTTA CCAGT GACGT
3201    CTTCC AGGAC TGCAA CAAGC TGGTG GACCC CGAGC CATAT CTGGA TGTCT
3251    GCATT TACGA CACCT GCTCC TGTGA GTCCA TTGGG GACTG CGCCG CATTC
3301    TGCGA CACCA TTGCT GCCTA TGCCC ACGTG TGTGC CCAGC ATGGC AAGGT
3351    GGTGA CCTGG AGGAC GGCCA CATTG TGCCC CCAGA GCTGC GAGGA GAGGA
3401    ATCTC CGGGA GAACG GGTAT GAGGC TGAGT GGCGC TATAA CAGCT GTGCA
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3851    GTGGC GGGGG ATCCG ACAAA ACTCA CACAT GCCCA CCGTG CCCAG CTCCA
3901    GAACT CCTGG GCGGA CCGTC AGTCT TCCTC TTCCC CCCAA AACCC AAGGA
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4001    TGAGC CACGA AGACC CTGAG GTCAA GTTCA ACTGG TACGT GGACG GCGTG
4051    GAGGT GCATA ATGCC AAGAC AAAGC CGCGG GAGGA GCAGT ACAAC AGCAC
4101    GTACC GTGTG GTCAG CGTCC TCACC GTCCT GGCCC AGGAC TGGCT GAATG
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VWF050 protein sequence (IHH triple mutant) (SEQ ID NO: 150)

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 151    SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
 201    ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
 251    EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
 301    YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTECPC
 351    VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD
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 451    LHNSLVKLKH GAGVAMDGQD IQLPLLKGDL RIQHTVTASV RLSYGEDLQM
 501    DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
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 701    CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
 751    AVLSSPLSHR SKRSLSCRPP MVKLVCPADN LRAEGLECTK TCQNYDLECM
 801    SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
 851    CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGECQ YVLVQDYCGS
 901    NPGTFRILVG NKGCSHPSVK CKKRVTILVE GGEIELFDGE VNVKRPMKDE
 951    THFEVVESGR YIILLLGKAL SVVWDRHLSI SVVLKQTYQE KVCGLCGNFD
1001    GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
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1201    VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP ISGGGGSGGG
1251    GSGGGGSGGG GSGGGGSGGG GSLVPRGSGG GGSGGGGSDK THTCPPCPAP
1301    ELLGGPSVFL FPPKPKDTLM ASRTPEVTCV VVDVSHEDPE VKFNWYVDGV
1351    EVHNAKTKPR EEQYNSTYRV VSVLTVLAQD WLNGKEYKCK VSNKALPAPI
1401    EKTISKAKGQ PREPQVYTLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE
1451    SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL
1501    HNAYTQKSLS LSPGK*
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VWF057 nucleotide sequence (SEQ ID NO: 151)

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 151  TACAG CTTTG CGGGA TACTG CAGTT ACCTC CTGGC AGGGG GCTGC CAGAA
 201  ACGCT CCTTC TCGAT TATTG GGGAC TTCCA GAATG GCAAG AGAGT GAGCC
 251  TCTCC GTGTA TCTTG GGGAA TTTTT TGACA TCCAT TTGTT TGTCA ATGGT
 301  ACCGT GACAC AGGGG GACCA AAGAG TCTCC ATGCC CTATG CCTCC AAAGG
 351  GCTGT ATCTA GAAAC TGAGG CTGGG TACTA CAAGC TGTCC GGTGA GGCCT
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1151  GTCCA GGGGA GTGCC TTGTC ACTGG TCAAT CCCAC TTCAA GAGCT TTGAC
1201  AACAG ATACT TCACC TTCAG TGGGA TCTGC CAGTA CCTGC TGGCC CGGGA
1251  TTGCC AGGAC CACTC CTTCT CCATT GTCAT TGAGA CTGTC CAGTG TGCTG
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1351  CTGCA CAACA GCCTT GTGAA ACTGA AGCAT GGGGC AGGAG TTGCC ATGGA
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1451  ATACA GTGAC GGCCT CCGTG CGCCT CAGCT ACGGG GAGGA CCTGC AGATG
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2251  GCTGT CCTCA GCAGT CCCCT GTCTC ATCGC AGCAA AAGGA GCCTA TCCTG
2301  TCGGC CCCCC ATGGT CAAGC TGGTG TGTCC CGCTG ACAAC CTGCG GGCTG
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2951  TGAAG CAGAC ATACC AGGAG AAAGT GTGTG GCCTG TGTGG GAATT TTGAT
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4051  ACGCC AGGAA CGAGC GAGTC CGCTA CACCG GAGAG TGGGC CAGGG AGCCC
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VWF057 protein sequence (SEQ ID NO: 152)

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1301    ESGPGTSTEP SEGSAPGSPA GSPTSTEEGT SESATPESGP GSEPATSGSE
1351    TPGTSESATP ESGPGSPAGS PTSTEEGSPA GSPTSTEEGA SSGGGGSGGG
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VWF058 nucleotide sequence (VWF034 with IHH mutation) (SEQ ID NO: 153)

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2301    TCGGC CCCCC ATGGT CAAGC TGGTG TGTCC CGCTG ACAAC CTGCG GGCTG
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2651    TGTTC CCCGG GGAGT GCCAG TACGT TCTGG TGCAG GATTA CTGCG GCAGT
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3151    ATGAA GCAGA CGATG GTGGA TTCCT CCTGT AGAAT CCTTA CCAGT GACGT
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4351    GCTAC CTCAG GGTCA GAGAC ACCCG GATCT CCGGC AGGCT CACCA ACCTC
4401    CACTG AGGAG GGCAC CAGCA CAGAA CCAAG CGAGG GCTCC GCACC CGGAA
4451    CAAGC ACTGA ACCCA GTGAG GGTTC AGCAC CCGGC TCTGA GCCGG CCACA
4501    AGTGG CAGTG AGACA CCCGG CACTT CAGAG AGTGC CACCC CCGAG AGTGG
4551    CCCAG GCACT AGTAC CGAGC CCTCT GAAGG CAGTG CGCCA GATTC TGGCG
4601    GTGGA GGTTC CGGTG GCGGG GGATC CGGTG GCGGG GGATC CGGTG GCGGG
4651    GGATC CGGTG GCGGG GGATC CCTGG TCCCC CGGGG CAGCG GAGGC GACAA
4701    AACTC ACACA TGCCC ACCGT GCCCA GCTCC AGAAC TCCTG GGCGG ACCGT
4751    CAGTC TTCCT CTTCC CCCCA AAACC CAAGG ACACC CTCAT GGCCT CCCGG

4801    ACCCC TGAGG TCACA TGCGT GGTGG TGGAC GTGAG CCACG AAGAC CCTGA
4851    GGTCA AGTTC AACTG GTACG TGGAC GGCGT GGAGG TGCAT AATGC CAAGA
4901    CAAAG CCGCG GGAGG AGCAG TACAA CAGCA CGTAC CGTGT GGTCA GCGTC
4951    CTCAC CGTCC TGGCC CAGGA CTGGC TGAAT GGCAA GGAGT ACAAG TGCAA
5001    GGTCT CCAAC AAAGC CCTCC CAGCC CCCAT CGAGA AAACC ATCTC CAAAG
5051    CCAAA GGGCA GCCCC GAGAA CCACA GGTGT ACACC CTGCC CCCAT CCCGC
5101    GATGA GCTGA CCAAG AACCA GGTCA GCCTG ACCTG CCTGG TCAAA GGCTT
5151    CTATC CCAGC GACAT CGCCG TGGAG TGGGA GAGCA ATGGG CAGCC GGAGA
5201    ACAAC TACAA GACCA CGCCT CCCGT GTTGG ACTCC GACGG CTCCT TCTTC
5251    CTCTA CAGCA AGCTC ACCGT GGACA AGAGC AGGTG GCAGC AGGGG AACGT
5301    CTTCT CATGC TCCGT GATGC ATGAG GCTCT GCACA ACGCC TACAC GCAGA
5351    AGAGC CTCTC CCTGT CTCCG GGTAA ATGA
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VWF058 protein sequence (VWF034 with IHH mutation) (SEQ ID NO: 154)

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   1  MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFVNTFDGSM
  51  YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
 101  TVTQGDQRVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
 151  SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
 201  ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
 251  EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
 301  YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTECPC
 351  VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD
 401  NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG
 451  LHNSLVKLKH GAGVAMDGQD IQLPLLKGDL RIQHTVTASV RLSYGEDLQM
 501  DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
 551  NAWKLHGDCQ DLQKQHSDPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
 601  PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCEL
 651  NCPKGQVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP PGLYMDERGD
 701  CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
 751  AVLSSPLSHR SKRSLSCRPP MVKLVCPADN LRAEGLECTK TCQNYDLECM
 801  SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
 851  CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGECQ YVLVQDYCGS
 901  NPGTFRILVG NKGCSHPSVK CKKRVTILVE GGEIELFDGE VNVKRPMKDE
 951  THFEVVESGR YIILLLGKAL SVVWDRHLSI SVVLKQTYQE KVCGLCGNFD
1001  GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
1051  MKQTMVDSSC RILTSDVFQD CNKLVDPEPY LDVCIYDTCS CESIGDCAAF
1101  CDTIAAYAHV CAQHGKVVTW RTATLCPQSC EERNLRENGY EAEWRYNSCA
1151  PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
1201  VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP ISGTSESATP
1251  ESGPGSEPAT SGSETPGTSE SATPESGPGS EPATSGSETP GTSESATPES
1301  GPGTSTEPSE GSAPGSPAGS PTSTEEGTSE SATPESGPGS EPATSGSETP
1351  GTSESATPES GPGSPAGSPT STEEGSPAGS PTSTEEGTST EPSEGSAPGT
1401  SESATPESGP GTSESATPES GPGTSESATP ESGPGSEPAT SGSETPGSEP
1451  ATSGSETPGS PAGSPTSTEE GTSTEPSEGS APGTSTEPSE GSAPGSEPAT
1501  SGSETPGTSE SATPESGPGT STEPSEGSAP DSGGGGSGGG GSGGGGSGGG
1551  GSGGGGSLVP RGSGGDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMASR
1601  TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
1651  LTVLAQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
1701  DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF
1751  LYSKLTVDKS RWQQGNVFSC SVMHEALHNA YTQKSLSLSP GK*
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FVIII 169 nucleotide sequence (SEQ ID NO: 155)

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   1  ATGCA AATAG AGCTC TCCAC CTGCT TCTTT CTGTG CCTTT TGCGA TTCTG
  51  CTTTA GTGCC ACCAG AAGAT ACTAC CTGGG TGCAG TGGAA CTGTC ATGGG
 101  ACTAT ATGCA AAGTG ATCTC GGTGA GCTGC CTGTG GACGC AAGAT TTCCT

 151  CCTAG AGTGC CAAAA TCTTT TCCAT TCAAC ACCTC AGTCG TGTAC AAAAA
 201  GACTC TGTTT GTAGA ATTCA CGGAT CACCT TTTCA ACATC GCTAA GCCAA
 251  GGCCA CCCTG GATGG GTCTG CTAGG TCCTA CCATC CAGGC TGAGG TTTAT
 301  GATAC AGTGG TCATT ACACT TAAGA ACATG GCTTC CCATC CTGTC AGTCT
 351  TCATG CTGTT GGTGT ATCCT ACTGG AAAGC TTCTG AGGGA GCTGA ATATG
 401  ATGAT CAGAC CAGTC AAAGG GAGAA AGAAG ATGAT AAAGT CTTCC CTGGT
 451  GGAAG CCATA CATAT GTCTG GCAGG TCCTG AAAGA GAATG GTCCA ATGGC
 501  CTCTG ACCCA CTGTG CCTTA CCTAC TCATA TCTTT CTCAT GTGGA CCTGG
 551  TAAAA GACTT GAATT CAGGC CTCAT TGGAG CCCTA CTAGT ATGTA GAGAA
 601  GGGAG TCTGG CCAAG GAAAA GACAC AGACC TTGCA CAAAT TTATA CTACT
 651  TTTTG CTGTA TTTGA TGAAG GGAAA AGTTG GCACT CAGAA ACAAA GAACT
 701  CCTTG ATGCA GGATA GGGAT GCTGC ATCTG CTCGG GCCTG GCCTA AAATG
 751  CACAC AGTCA ATGGT TATGT AAACA GGTCT CTGCC AGGTC TGATT GGATG
 801  CCACA GGAAA TCAGT CTATT GGCAT GTGAT TGGAA TGGGC ACCAC TCCTG
 851  AAGTG CACTC AATAT TCCTC GAAGG TCACA CATTT CTTGT GAGGA ACCAT
 901  CGCCA GGCTA GCTTG GAAAT CTCGC CAATA ACTTT CCTTA CTGCT CAAAC
 951  ACTCT TGATG GACCT TGGAC AGTTT CTACT GTTTT GTCAT ATCTC TTCCC
1001  ACCAA CATGA TGGCA TGGAA GCTTA TGTCA AAGTA GACAG CTGTC CAGAG
1051  GAACC CCAAC TACGA ATGAA AAATA ATGAA GAAGC GGAAG ACTAT GATGA
1101  TGATC TTACT GATTC TGAAA TGGAT GTGGT CAGGT TTGAT GATGA CAACT
1151  CTCCT TCCTT TATCC AAATT CGCTC AGTTG CCAAG AAGCA TCCTA AAACT
1201  TGGGT ACATT ACATT GCTGC TGAAG AGGAG GACTG GGACT ATGCT CCCTT
1251  AGTCC TCGCC CCCGA TGACA GAAGT TATAA AAGTC AATAT TTGAA CAATG
1301  GCCCT CAGCG GATTG GTAGG AAGTA CAAAA AAGTC CGATT TATGG CATAC
1351  ACAGA TGAAA CCTTT AAGAC TCGTG AAGCT ATTCA GCATG AATCA GGAAT
1401  CTTGG GACCT TTACT TTATG GGGAA GTTGG AGACA CACTG TTGAT TATAT
1451  TTAAG AATCA AGCAA GCAGA CCATA TAACA TCTAC CCTCA CGGAA TCACT
1501  GATGT CCGTC CTTTG TATTC AAGGA GATTA CCAAA AGGTG TAAAA CATTT
1551  GAAGG ATTTT CCAAT TCTGC CAGGA GAAAT ATTCA AATAT AAATG GACAG
1601  TGACT GTAGA AGATG GGCCA ACTAA ATCAG ATCCT CGGTG CCTGA CCCGC
1651  TATTA CTCTA GTTTC GTTAA TATGG AGAGA GATCT AGCTT CAGGA CTCAT
1701  TGGCC CTCTC CTCAT CTGCT ACAAA GAATC TGTAG ATCAA AGAGG AAACC
1751  AGATA ATGTC AGACA AGAGG AATGT CATCC TGTTT TCTGT ATTTG ATGAG
1801  AACCG AAGCT GGTAC CTCAC AGAGA ATATA CAACG CTTTC TCCCC AATCC
1851  AGCTG GAGTG CAGCT TGAGG ATCCA GAGTT CCAAG CCTCC AACAT CATGC
1901  ACAGC ATCAA TGGCT ATGTT TTTGA TAGTT TGCAG TTGTC AGTTT GTTTG
1951  CATGA GGTGG CATAC TGGTA CATTC TAAGC ATTGG AGCAC AGACT GACTT
2001  CCTTT CTGTC TTCTT CTCTG GATAT ACCTT CAAAC ACAAA ATGGT CTATG
2051  AAGAC ACACT CACCC TATTC CCATT CTCAG GAGAA ACTGT CTTCA TGTCG
2101  ATGGA AAACC CAGGT CTATG GATTC TGGGG TGCCA CAACT CAGAC TTTCG
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2101    ATGGA AAACC CAGGT CTATG GATTC TGGGG TGCGA CAACT CACAC TTTCC
2151    GAACA GAGGC ATGAC CGCCT TACTG AAGGT TTCTA GTTGT GACAA GAACA
2201    CTGGT GATTA TTACG AGGAC AGTTA TGAAG ATATT TCAGC ATACT TGCTG
2251    AGTAA AAACA ATGCC ATTGA ACCAA GAAGC TTCTC TCAAA ACGGC GCGCC
2301    AGGTA CCTCA GAGTC TGCTA CCCCC GAGTC AGGGC CAGGA TCAGA GCCAG
2351    CCACC TCCGG GTCTG AGACA CCCGG GACTT CCGAG AGTGC CACCC CTGAG
2401    TCCGG ACCCG GGTCC GAGCC CGCCA CTTCC GGCTC CGAAA CTCCC GGCAC
2451    AAGCG AGAGC GCTAC CCCAG AGTCA GGACC AGGAA CATCT ACAGA GCCCT
2501    CTGAA GGCTC CGCTC CAGGG TCCCC AGCCG GCAGT CCCAC TAGCA CCGAG
2551    GAGGG AACCT CTGAA AGCGC CACAC CCGAA TCAGG GCCAG GGTCT GAGCC
2601    TGCTA CCAGC GGCAG CGAGA CACCA GGCAC CTCTG AGTCC GCCAC ACCAG
2651    AGTCC GGACC CGGAT CTCCC GCTGG GAGCC CCACC TCCAC TGAGG AGGGA
2701    TCTCC TGCTG GCTCT CCAAC ATCTA CTGAG GAAGG TACCT CAACC GAGCC
2751    ATCCG AGGGA TCAGC TCCCG GCACC TCAGA GTCGG CAACC CCGGA GTCTG
2801    GACCC GGAAC TTCCG AAAGT GCCAC ACCAG AGTCC GGTCC CGGGA CTTCA
2851    GAATC AGCAA CACCC GAGTC CGGCC CTGGG TCTGA ACCCG CCACA AGTGG
2901    TAGTG AGACA CCAGG ATCAG AACCT GCTAC CTCAG GGTCA GAGAC ACCCG
2951    GATCT CCGGC AGGCT CACCA ACCTC CACTG AGGAG GGCAC CAGCA CAGAA
3001    CCAAG CGAGG GCTCC GCACC CGGAA CAAGC ACTGA ACCCA GTGAG GGTTC
3051    AGCAC CCGGC TCTGA GCCGG CCACA AGTGG CAGTG AGACA CCCGG CACTT

3101    CAGAG AGTGC CACCC CCGAG AGTGG CCCAG GCACT AGTAC CGAGC CCTCT
3151    GAAGG CAGTG CGCCA GCCTC GAGCC CACCA GTCTT GAAAC GCCAT CAAGC
3201    TGAAA TAACT CGTAC TACTC TTCAG TCAGA TCAAG AGGAA ATCGA TTATG
3251    ATGAT ACCAT ATCAG TTGAA ATGAA GAAGG AAGAT TTTGA CATTT ATGAT
3301    GAGGA TGAAA ATCAG AGCCC CCGCA GCTTT CAAAA GAAAA CACGA CACTA
3351    TTTTA TTGCT GCAGT GGAGA GGCTC TGGGA TTATG GGATG AGTAG CTCCC
3401    CACAT GTTCT AAGAA ACAGG GCTCA GAGTG GCAGT GTCCC TCAGT TCAAG
3451    AAAGT TGTTT TCCAG GAATT TACTG ATGGC TCCTT TACTC AGCCC TTATA
3501    CCGTG GAGAA CTAAA TGAAC ATTTG GGACT CCTGG GGCCA TATAT AAGAG
3551    CAGAA GTTGA AGATA ATATC ATGGT AACTT TCAGA AATCA GGCCT CTCGT
3601    CCCTA TTCCT TCTAT TCTAG CCTTA TTTCT TATGA GGAAG ATCAG AGGCA
3651    AGGAG CAGAA CCTAG AAAAA ACTTT GTCAA GCCTA ATGAA ACCAA AACTT
3701    ACTTT TGGAA AGTGC AACAT CATAT GGCAC CCACT AAAGA TGAGT TTGAC
3751    TGCAA AGCCT GGGCT TATTT CTCTG ATGTT GACCT GGAAA AAGAT GTGCA
3801    CTCAG GCCTG ATTGG ACCCC TTCTG GTCTG CCACA CTAAC ACACT GAACC
3851    CTGCT CATGG GAGAC AAGTG ACAGT ACAGG AATTT GCTCT GTTTT TCACC
3901    ATCTT TGATG AGACC AAAAG CTGGT ACTTC ACTGA AAATA TGGAA AGAAA
3951    CTGCA GGGCT CCCTG CAATA TCCAG ATGGA AGATC CCACT TTTAA AGAGA
4001    ATTAT CGCTT CCATG CAATC AATGG CTACA TAATG GATAC ACTAC CTGGC
4051    TTAGT AATGG CTCAG GATCA AAGGA TTCGA TGGTA TCTGC TCAGC ATGGG
4101    CAGCA ATGAA AACAT CCATT CTATT CATTT CAGTG GACAT GTGTT CACTG
4151    TACGA AAAAA AGAGG AGTAT AAAAT GGCAC TGTAC AATCT CTATC CAGGT
4201    GTTTT TGAGA CAGTG GAAAT GTTAC CATCC AAAGC TGGAA TTTGG CGGGT
4251    GGAAT GCCTT ATTGG CGAGC ATCTA CATGC TGGGA TGAGC ACACT TTTTC
4301    TGGTG TACAG CAATA AGTGT CAGAC TCCCC TGGGA ATGGC TTCTG GACAC
4351    ATTAG AGATT TTCAG ATTAC AGCTT CAGGA CAATA TGGAC AGTGG GCCCC
4401    AAAGC TGGCC AGACT TCATT ATTCC GGATC AATCA ATGCC TGGAG CACCA
4451    AGGAG CCCTT TTCTT GGATC AAGGT GGATC TGTTG GCACC AATGA TTATT
4501    CACGG CATCA AGACC CAGGG TGCCC GTCAG AAGTT CTCCA GCCTC TACAT
4551    CTCTC AGTTT ATCAT CATGT ATAGT CTTGA TGGGA AGAAG TGGCA GACTT
4601    ATCGA GGAAA TTCCA CTGGA ACCTT AATGG TCTTC TTTGG CAATG TGGAT
4651    TCATC TGGGA TAAAA CACAA TATTT TTAAC CCTCC AATTA TTGCT CGATA
4701    CATCC GTTTG CACCC AACTC ATTAT AGCAT TCGCA GCACT CTTCG CATGG
4751    AGTTG ATGGG CTGTG ATTTA AATAG TTGCA GCATG CCATT GGGAA TGGAG
4801    AGTAA AGCAA TATCA GATGC ACAGA TTACT GCTTC ATCCT ACTTT ACCAA
4851    TATGT TTGCC ACCTG GTCTC CTTCA AAAGC TCGAC TTCAC CTCCA AGGGA
4901    GGAGT AATGC CTGGA GACCT CAGGT GAATA ATCCA AAAGA GTGGC TGCAA
4951    GTGGA CTTCC AGAAG ACAAT GAAAG TCACA GGAGT AACTA CTCAG GGAGT
5001    AAAAT CTCTG CTTAC CAGCA TGTAT GTGAA GGAGT TCCTC ATCTC CAGCA
5051    GTCAA GATGG CCATC AGTGG ACTCT CTTTT TTCAG AATGG CAAAG TAAAG
5101    GTTTT TCAGG GAAAT CAAGA CTCCT TCACA CCTGT GGTGA ACTCT CTAGA
5151    CCCAC CGTTA CTGAC TCGCT ACCTT CGAAT TCACC CCCAG AGTTG GGTGC
5201    ACCAG ATTGC CCTGA GGATG GAGGT TCTGG GCTGC GAGGC ACAGG ACCTC
5251    TACGA CAAAA CTCAC ACATG CCCAC CGTGC CCAGC TCCAG AACTC CTGGG
5301    CGGAC CGTCA GTCTT CCTCT TCCCC CCAAA ACCCA AGGAC ACCCT CATGA
5351    TCTCC CGGAC CCCTG AGGTC ACATG CGTGG TGGTG GACGT GAGCC ACGAA
5401    GACCC TGAGG TCAAG TTCAA CTGGT ACGTG GACGG CGTGG AGGTG CATAA
5451    TGCCA AGACA AAGCC GCGGG AGGAG CAGTA CAACA GCACG TACCG TGTGG
5501    TCAGC GTCCT CACCG TCCTG CACCA GGACT GGCTG AATGG CAAGG AGTAC
5551    AAGTG CAAGG TCTCC AACAA AGCCC TCCCA GCCCC CATCG AGAAA ACCAT
5601    CTCCA AAGCC AAAGG GCAGC CCCGA GAACC ACAGG TGTAC ACCCT GCCCC
5651    CATCC CGGGA TGAGC TGACC AAGAA CCAGG TCAGC CTGAC CTGCC TGGTC
5701    AAAGG CTTCT ATCCC AGCGA CATCG CCGTG GAGTG GGAGA GCAAT GGGCA
5751    GCCGG AGAAC AACTA CAAGA CCACG CCTCC CGTGT TGGAC TCCGA CGGCT
5801    CCTTC TTCCT CTACA GCAAG CTCAC CGTGG ACAAG AGCAG GTGGC AGCAG
5851    GGGAA CGTCT TCTCA TGCTC CGTGA TGCAT GAGGC TCTGC ACAAC CACTA
5901    CACGC AGAAG AGCCT CTCCC TGTCT CCGGG TAAAT GA
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FVIII 169 protein sequence(SEQ ID NO: 70)

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   1    MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP
  51    PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101    DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 151    GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201    GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
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 201    GSLAREKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251    HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301    RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351    EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401    WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451    TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 501    DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551    YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601    NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651    HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 701    MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 751    SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSESATPE
 801    SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
 851    EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP AGSPTSTEEG
 901    SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
 951    ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
1001    PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPGTSTEPS
1051    EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
1101    EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVPQFK
1151    KVVFQEFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
1201    PYSFYSSLIS YEEDQRQGAE PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
1251    CKAWAYFSDV DLEKDVHSGL IGPLLVCHTN TLNPAHGRQV TVQEFALFFT
1301    IFDETKSWYF TENMERNCRA PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
1351    LVMAQDQRIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
1401    VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
1451    IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
1501    HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
1551    SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
1601    SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
1651    VDFQKTMKVT GVTTQGVKSL LTSMYVKEFL ISSSQDGHQW TLFFQNGKVK
1701    VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
1751    YDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
1801    DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
1851    KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV
1901    KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
1951    GNVFSCSVMH EALHNHYTQK SLSLSPGK*
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FVIII 263 nucleotide sequence (IHH triple mutant) (SEQ ID NO: 156)

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   1    ATGCA AATAG AGCTC TCCAC CTGCT TCTTT CTGTG CCTTT TGCGA TTCTG
  51    CTTTA GTGCC ACCAG AAGAT ACTAC CTGGG TGCAG TGGAA CTGTC ATGGG
 101    ACTAT ATGCA AGGCG CGCCA ACATC AGAGA GCGCC ACCCC TGAAA GTGGT
 151    CCCGG GAGCG AGCCA GCCAC ATCTG GGTCG GAAAC GCCAG GCACA AGTGA
 201    GTCTG CAACT CCCGA GTCCG GACCT GGCTC CGAGC CTGCC ACTAG CGGCT
 251    CCGAG ACTCC GGGAA CTTCC GAGAG CGCTA CACCA GAAAG CGGAC CCGGA
 301    ACCAG TACCG AACCT AGCGA GGGCT CTGCT CCGGG CAGCC CAGCC GGCTC
 351    TCCTA CATCC ACGGA GGAGG GCACT TCCGA ATCCG CCACC CCGGA GTCAG
 401    GGCCA GGATC TGAAC CCGCT ACCTC AGGCA GTGAG ACGCC AGGAA CGAGC
 451    GAGTC CGCTA CACCG GAGAG TGGGC CAGGG AGCCC TGCTG GATCT CCTAC
 501    GTCCA CTGAG GAAGG GTCAC CAGCG GGCTC GCCCA CCAGC ACTGA AGAAG
 551    GTGCC TCGAG CAGTG ATCTC GGTGA GCTGC CTGTG GACGC AAGAT TTCCT
 601    CCTAG AGTGC CAAAA TCTTT TCCAT TCAAC ACCTC AGTCG TGTAC AAAAA
 651    GACTC TGTTT GTAGA ATTCA CGGAT CACCT TTTCA ACATC GCTAA GCCAA
 701    GGCCA CCCTG GATGG GTCTG CTAGG TCCTA CCATC CAGGC TGAGG TTTAT

 751    GATAC AGTGG TCATT ACACT TAAGA ACATG GCTTC CCATC CTGTC AGTCT
 801    TCATG CTGTT GGTGT ATCCT ACTGG AAAGC TTCTG AGGGA GCTGA ATATG
 851    ATGAT CAGAC CAGTC AAAGG GAGAA AGAAG ATGAT AAAGT CTTCC CTGGT
 901    GGAAG CCATA CATAT GTCTG GCAGG TCCTG AAAGA GAATG GTCCA ATGGC
 951    CTCTG ACCCA CTGTG CCTTA CCTAC TCATA TCTTT CTCAT GTGGA CCTGG
1001    TAAAA GACTT GAATT CAGGC CTCAT TGGAG CCCTA CTAGT ATGTA GAGAA
1051    GGGAG TCTGG CCAAG GAAAA GACAC AGACC TTGCA CAAAT TTATA CTACT
1101    TTTTG CTGTA TTTGA TGAAG GGAAA AGTTG GCACT CAGAA ACAAA GAACT
1151    CCTTG ATGCA GGATA GGGAT GCTGC ATCTG CTCGG GCCTG GCCTA AAATG
1201    CACAC AGTCA ATGGT TATGT AAACA GGTCT CTGCC AGGTC TGATT GGATG
1251    CCACA GGAAA TCAGT CTATT GGCAT GTGAT TGGAA TGGGC ACCAC TCCTG
1301    AAGTG CACTC AATAT TCCTC GAAGG TCACA CATTT CTTGT GAGGA ACCAT
1351    CGCCA GGCTA GCTTG GAAAT CTCGC CAATA ACTTT CCTTA CTGCT CAAAC
1401    ACTCT TGATG GACCT TGGAC AGTTT CTACT GTTTT GTCAT ATCTC TTCCC
1451    ACCAA CATGA TGGCA TGGAA GCTTA TGTCA AAGTA GACAG CTGTC CAGAG
1501    GAACC CCAAC TACGA ATGAA AAATA ATGAA GAAGC GGAAG ACTAT GATGA
1551    TGATC TTACT GATTC TGAAA TGGAT GTGGT CAGGT TTGAT GATGA CAACT
1601    CTCCT TCCTT TATCC AAATT CGCTC AGTTG CCAAG AAGCA TCCTA AAACT
1651    TGGGT ACATT ACATT GCTGC TGAAG AGGAG GACTG GGACT ATGCT CCCTT
1701    AGTCC TCGCC CCCGA TGACA GAAGT TATAA AAGTC AATAT TTGAA CAATG
1751    GCCCT CAGCG GATTG GTAGG AAGTA CAAAA AAGTC CGATT TATGG CATAC
1801    ACAGA TGAAA CCTTT AAGAC TCGTG AAGCT ATTCA GCATG AATCA GGAAT
1851    CTTGG GACCT TTACT TTATG GGGAA GTTGG AGACA CACTG TTGAT TATAT
1901    TTAAG AATCA AGCAA GCAGA CCATA TAACA TCTAC CCTCA CGGAA TCACT
1951    GATGT CCGTC CTTTG TATTC AAGGA GATTA CCAAA AGGTG TAAAA CATTT
2001    GAAGG ATTTT CCAAT TCTGC CAGGA GAAAT ATTCA AATAT AAATG GACAG
2051    TGACT GTAGA AGATG GCCCA ACTAA ATCAG ATCCT CGGTG CCTGA CCCGC
2101    TATTA CTCTA GTTTC GTTAA TATGG AGAGA GATCT AGCTT CAGGA CTCAT
2151    TGGCC CTCTC CTCAT CTGCT ACAAA GAATC TGTAG ATCAA AGAGG AAACC
2201    AGATA ATGTC AGACA AGAGG AATGT CATCC TGTTT TCTGT ATTTG ATGAG
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2251    AACCG AAGCT GGTAC CTCAC AGAGA ATATA CAACG CTTTC TCCCC AATCC
2301    AGCTG GAGTG CAGCT TGAGG ATCCA GAGTT CCAAG CCTCC AACAT CATGC
2351    ACAGC ATCAA TGGCT ATGTT TTTGA TAGTT TGCAG TTGTC AGTTT GTTTG
2401    CATGA GGTGG CATAC TGGTA CATTC TAAGC ATTGG AGCAC AGACT GACTT
2451    CCTTT CTGTC TTCTT CTCTG GATAT ACCTT CAAAC ACAAA ATGGT CTATG
2501    AAGAC ACACT CACCC TATTC CCATT CTCAG GAGAA ACTGT CTTCA TGTCG
2551    ATGGA AAACC CAGGT CTATG GATTC TGGGG TGCCA CAACT CAGAC TTTCG
2601    GAACA GAGGC ATGAC CGCCT TACTG AAGGT TTCTA GTTGT GACAA GAACA
2651    CTGGT GATTA TTACG AGGAC AGTTA TGAAG ATATT TCAGC ATACT TGCTG
2701    AGTAA AAACA ATGCC ATTGA ACCAA GAAGC TTCTC TCAAA ACGGC GCGCC
2751    AGGTA CCTCA GAGTC TGCTA CCCCC GAGTC AGGGC CAGGA TCAGA GCCAG
2801    CCACC TCCGG GTCTG AGACA CCCGG GACTT CCGAG AGTGC CACCC CTGAG
2851    TCCGG ACCCG GGTCC GAGCC CGCCA CTTCC GGCTC CGAAA CTCCC GGCAC
2901    AAGCG AGAGC GCTAC CCCAG AGTCA GGACC AGGAA CATCT ACAGA GCCCT
2951    CTGAA GGCTC CGCTC CAGGG TCCCC AGCCG GCAGT CCCAC TAGCA CCGAG
3001    GAGGG AACCT CTGAA AGCGC CACAC CCGAA TCAGG GCCAG GGTCT GAGCC
3051    TGCTA CCAGC GGCAG CGAGA CACCA GGCAC CTCTG AGTCC GCCAC ACCAG
3101    AGTCC GGACC CGGAT CTCCC GCTGG GAGCC CCACC TCCAC TGAGG AGGGA
3151    TCTCC TGCTG GCTCT CCAAC ATCTA CTGAG GAAGG TACCT CAACC GAGCC
3201    ATCCG AGGGA TCAGC TCCCG GCACC TCAGA GTCGG CAACC CCGGA GTCTG
3251    GACCC GGAAC TTCCG AAAGT GCCAC ACCAG AGTCC GGTCC CGGGA CTTCA
3301    GAATC AGCAA CACCC GAGTC CGGCC CTGGG TCTGA ACCCG CCACA AGTGG
3351    TAGTG AGACA CCAGG ATCAG AACCT GCTAC CTCAG GGTCA GAGAC ACCCG
3401    GATCT CCGGC AGGCT CACCA ACCTC CACTG AGGAG GGCAC CAGCA CAGAA
3451    CCAAG CGAGG GCTCC GCACC CGGAA CAAGC ACTGA ACCCA GTGAG GGTTC
3501    AGCAC CCGGC TCTGA GCCGG CCACA AGTGG CAGTG AGACA CCCGG CACTT
3551    CAGAG AGTGC CACCC CCGAG AGTGG CCCAG GCACT AGTAC CGAGC CCTCT
3601    GAAGG CAGTG CGCCA GCCTC GAGCC CACCA GTCTT GAAAC GCCAT CAAGC
3651    TGAAA TAACT CGTAC TACTC TTCAG TCAGA TCAAG AGGAA ATCGA TTATG

3701    ATGAT ACCAT ATCAG TTGAA ATGAA GAAGG AAGAT TTTGA CATTT ATGAT
3751    GAGGA TGAAA ATCAG AGCCC CCGCA GCTTT CAAAA GAAAA CACGA CACTA
3801    TTTTA TTGCT GCAGT GGAGA GGCTC TGGGA TTATG GGATG AGTAG CTCCC
3851    CACAT GTTCT AAGAA ACAGG GCTCA GAGTG GCAGT GTCCC TCAGT TCAAG
3901    AAAGT TGTTT TCCAG GAATT TACTG ATGGC TCCTT TACTC AGCCC TTATA
3951    CCGTG GAGAA CTAAA TGAAC ATTTG GGACT CCTGG GGCCA TATAT AAGAG
4001    CAGAA GTTGA AGATA ATATC ATGGT AACTT TCAGA AATCA GGCCT CTCGT
4051    CCCTA TTCCT TCTAT TCTAG CCTTA TTTCT TATGA GGAAG ATCAG AGGCA
4101    AGGAG CAGAA CCTAG AAAAA ACTTT GTCAA GCCTA ATGAA ACCAA AACTT
4151    ACTTT TGGAA AGTGC AACAT CATAT GGCAC CCACT AAAGA TGAGT TTGAC
4201    TGCAA AGCCT GGGCT TATTT CTCTG ATGTT GACCT GGAAA AAGAT GTGCA
4251    CTCAG GCCTG ATTGG ACCCC TTCTG GTCTG CCACA CTAAC ACACT GAACC
4301    CTGCT CATGG GAGAC AAGTG ACAGT ACAGG AATTT GCTCT GTTTT TCACC
4351    ATCTT TGATG AGACC AAAAG CTGGT ACTTC ACTGA AAATA TGGAA AGAAA
4401    CTGCA GGGCT CCCTG CAATA TCCAG ATGGA AGATC CCACT TTTAA AGAGA
4451    ATTAT CGCTT CCATG CAATC AATGG CTACA TAATG GATAC ACTAC CTGGC
4501    TTAGT AATGG CTCAG GATCA AAGGA TTCGA TGGTA TCTGC TCAGC ATGGG
4551    CAGCA ATGAA AACAT CCATT CTATT CATTT CAGTG GACAT GTGTT CACTG
4601    TACGA AAAAA AGAGG AGTAT AAAAT GGCAC TGTAC AATCT CTATC CAGGT
4651    GTTTT TGAGA CAGTG GAAAT GTTAC CATCC AAAGC TGGAA TTTGG CGGGT
4701    GGAAT GCCTT ATTGG CGAGC ATCTA CATGC TGGGA TGAGC ACACT TTTTC
4751    TGGTG TACAG CAATA AGTGT CAGAC TCCCC TGGGA ATGGC TTCTG GACAC
4801    ATTAG AGATT TTCAG ATTAC AGCTT CAGGA CAATA TGGAC AGTGG GCCCC
4851    AAAGC TGGCC AGACT TCATT ATTCC GGATC AATCA ATGCC TGGAG CACCA
4901    AGGAG CCCTT TTCTT GGATC AAGGT GGATC TGTTG GCACC AATGA TTATT
4951    CACGG CATCA AGACC CAGGG TGCCC GTCAG AAGTT CTCCA GCCTC TACAT
5001    CTCTC AGTTT ATCAT CATGT ATAGT CTTGA TGGGA AGAAG TGGCA GACTT
5051    ATCGA GGAAA TTCCA CTGGA ACCTT AATGG TCTTC TTTGG CAATG TGGAT
5101    TCATC TGGGA TAAAA CACAA TATTT TTAAC CCTCC AATTA TTGCT CGATA
5151    CATCC GTTTG CACCC AACTC ATTAT AGCAT TCGCA GCACT CTTCG CATGG
5201    AGTTG ATGGG CTGTG ATTTA AATAG TTGCA GCATG CCATT GGGAA TGGAG
5251    AGTAA AGCAA TATCA GATGC ACAGA TTACT GCTTC ATCCT ACTTT ACCAA
5301    TATGT TTGCC ACCTG GTCTC CTTCA AAAGC TCGAC TTCAC CTCCA AGGGA
5351    GGAGT AATGC CTGGA GACCT CAGGT GAATA ATCCA AAAGA GTGGC TGCAA
5401    GTGGA CTTCC AGAAG ACAAT GAAAG TCACA GGAGT AACTA CTCAG GGAGT
5451    AAAAT CTCTG CTTAC CAGCA TGTAT GTGAA GGAGT TCCTC ATCTC CAGCA
5501    GTCAA GATGG CCATC AGTGG ACTCT CTTTT TTCAG AATGG CAAAG TAAAG
5551    GTTTT TCAGG GAAAT CAAGA CTCCT TCACA CCTGT GGTGA ACTCT CTAGA
5601    CCCAC CGTTA CTGAC TCGCT ACCTT CGAAT TCACC CCCAG AGTTG GGTGC
5651    ACCAG ATTGC CCTGA GGATG GAGGT TCTGG GCTGC GAGGC ACAGG ACCTC
5701    TACGA CAAAA CTCAC ACATG CCCAC CGTGC CCAGC TCCAG AACTC CTGGG
5751    CGGAC CGTCA GTCTT CCTCT TCCCC CCAAA ACCCA AGGAC ACCCT CATGG
5801    CCTCC CGGAC CCCTG AGGTC ACATG CGTGG TGGTG GACGT GAGCC ACGAA
5851    GACCC TGAGG TCAAG TTCAA CTGGT ACGTG GACGG CGTGG AGGTG CATAA
5901    TGCCA AGACA AAGCC GCGGG AGGAG CAGTA CAACA GCACG TACCG TGTGG
5951    TCAGC GTCCT CACCG TCCTG GCCCA GGACT GGCTG AATGG CAAGG AGTAC
6001    AAGTG CAAGG TCTCC AACAA AGCCC TCCCA GCCCC CATCG AGAAA ACCAT
6051    CTCCA AAGCC AAAGG GCAGC CCCGA GAACC ACAGG TGTAC ACCCT GCCCC
6101    CATCC CGCGA TGAGC TGACC AAGAA CCAGG TCAGC CTGAC CTGCC TGGTC
6151    AAAGG CTTCT ATCCC AGCGA CATCG CCGTG GAGTG GGAGA GCAAT GGGCA
6201    GCCGG AGAAC AACTA CAAGA CCACG CCTCC CGTGT TGGAC TCCGA CGGCT
6251    CCTTC TTCCT CTACA GCAAG CTCAC CGTGG ACAAG AGCAG GTGGC AGCAG
6301    GGGAA CGTCT TCTCA TGCTC CGTGA TGCAT GAGGC TCTGC ACAAC GCCTA
6351    CACGC AGAAG AGCCT CTCCC TGTCT CCGGG TAAAT GA
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FVIII 263 protein sequence(IHH triple mutant) (SEQ ID NO: 157)

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   1  MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQGAP TSESATPESG
  51  PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG

 101  TSTEPSEGSA PGSPAGSPTS TEEGTSESAT PESGPGSEPA TSGSETPGTS
 151  ESATPESGPG SPAGSPTSTE EGSPAGSPTS TEEGASSSDL GELPVDARFP
 201  PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 251  DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 301  GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 351  GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 401  HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 451  RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 501  EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 551  WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 601  TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 651  DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 701  YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 751  NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 801  HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 851  MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 901  SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSESATPE
 951  SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
1001  EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP AGSPTSTEEG
1051  SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
1101  ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
1151  PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPGTSTEPS
1201  EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
1251  EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVPQFK
1301  KVVFQEFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
1351  PYSFYSSLIS YEEDQRQGAE PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
1401  CKAWAYFSDV DLEKDVHSGL IGPLLVCHTN TLNPAHGRQV TVQEFALFFT
1451  IFDETKSWYF TENMERNCRA PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
1501  LVMAQDQRIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
1551  VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
1601  IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
1651  HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
1701  SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
1751  SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
1801  VDFQKTMKVT GVTTQGVKSL LTSMYVKEFL ISSSQDGHQW TLFFQNGKVK
1851  VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
1901  YDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMASRTPEV TCVVVDVSHE
1951  DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL AQDWLNGKEY
2001  KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV
2051  KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
2101  GNVFSCSVMH EALHNAYTQK SLSLSPGK*
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FVIII 282 nucleotide sequence(SEQ ID NO: 158)

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  51  CTTTA GTGCC ACCAG AAGAT ACTAC CTGGG TGCAG TGGAA CTGTC ATGGG
 101  ACTAT ATGCA AAGTG ATCTC GGTGA GCTGC CTGTG GACGC AAGAT TTCCT
 151  CCTAG AGTGC CAAAA TCTTT TCCAT TCAAC ACCTC AGTCG TGTAC AAAAA
 201  GACTC TGTTT GTAGA ATTCA CGGAT CACCT TTTCA ACATC GCTAA GCCAA
 251  GGCCA CCCTG GATGG GTCTG CTAGG TCCTA CCATC CAGGC TGAGG TTTAT
 301  GATAC AGTGG TCATT ACACT TAAGA ACATG GCTTC CCATC CTGTC AGTCT
 351  TCATG CTGTT GGTGT ATCCT ACTGG AAAGC TTCTG AGGGA GCTGA ATATG
 401  ATGAT CAGAC CAGTC AAAGG GAGAA AGAAG ATGAT AAAGT CTTCC CTGGT
 451  GGAAG CCATA CATAT GTCTG GCAGG TCCTG AAAGA GAATG GTCCA ATGGC
 501  CTCTG ACCCA CTGTG CCTTA CCTAC TCATA TCTTT CTCAT GTGGA CCTGG
 551  TAAAA GACTT GAATT CAGGC CTCAT TGGAG CCCTA CTAGT ATGTA GAGAA
 601  GGGAG TCTGG CCAAG GAAAA GACAC AGACC TTGCA CAAAT TTATA CTACT
 651  TTTTG CTGTA TTTGA TGAAG GGAAA AGTTG GCACT CAGAA ACAAA GAACT
 701  CCTTG ATGCA GGATA GGGAT GCTGC ATCTG CTCGG GCCTG GCCTA AAATG

 751  CACAC AGTCA ATGGT TATGT AAACA GGTCT CTGCC AGGTC TGATT GGATG
 801  CCACA GGAAA TCAGT CTATT GGCAT GTGAT TGGAA TGGGC ACCAC TCCTG
 851  AAGTG CACTC AATAT TCCTC GAAGG TCACA CATTT CTTGT GAGGA ACCAT
 901  CGCCA GGCTA GCTTG GAAAT CTCGC CAATA ACTTT CCTTA CTGCT CAAAC
 951  ACTCT TGATG GACCT TGGAC AGTTT CTACT GTTTT GTCAT ATCTC TTCCC
1001  ACCAA CATGA TGGCA TGGAA GCTTA TGTCA AAGTA GACAG CTGTC CAGAG
1051  GAACC CCAAC TACGA ATGAA AAATA ATGAA GAAGC GGAAG ACTAT GATGA
1101  TGATC TTACT GATTC TGAAA TGGAT GTGGT CAGGT TTGAT GATGA CAACT
1151  CTCCT TCCTT TATCC AAATT CGCTC AGTTG CCAAG AAGCA TCCTA AAACT
1201  TGGGT ACATT ACATT GCTGC TGAAG AGGAG GACTG GGACT ATGCT CCCTT
1251  AGTCC TCGCC CCCGA TGACA GAAGT TATAA AAGTC AATAT TTGAA CAATG
1301  GCCCT CAGCG GATTG GTAGG AAGTA CAAAA AAGTC CGATT TATGG CATAC
1351  ACAGA TGAAA CCTTT AAGAC TCGTG AAGCT ATTCA GCATG AATCA GGAAT
1401  CTTGG GACCT TTACT TTATG GGGAA GTTGG AGACA CACTG TTGAT TATAT
1451  TTAAG AATCA AGCAA GCAGA CCATA TAACA TCTAC CCTCA CGGAA TCACT
1501  GATGT CCGTC CTTTG TATTC AAGGA GATTA CCAAA AGGTG TAAAA CATTT
1551  GAAGG ATTTT CCAAT TCTGC CAGGA GAAAT ATTCA AATAT AAATG GACAG
1601  TGACT GTAGA AGATG GGCCA ACTAA ATCAG ATCCT CGGTG CCTGA CCCGC
1651  TATTA CTCTA GTTTC GTTAA TATGG AGAGA GATCT AGCTT CAGGA CTCAT
1701  TCCCC CTCTC CTCAT CTCCT AGAAA GAATC TCTAC ATGAA AGACC AAACC
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1701    TGGCC CTCTC CTCAT CTGCT ACAAA GAATC TGTAG ATCAA AGAGG AAACC
1751    AGATA ATGTC AGACA AGAGG AATGT CATCC TGTTT TCTGT ATTTG ATGAG
1801    AACCG AAGCT GGTAC CTCAC AGAGA ATATA CAACG CTTTC TCCCC AATCC
1851    AGCTG GAGTG CAGCT TGAGG ATCCA GAGTT CCAAG CCTCC AACAT CATGC
1901    ACAGC ATCAA TGGCT ATGTT TTTGA TAGTT TGCAG TTGTC AGTTT GTTTG
1951    CATGA GGTGG CATAC TGGTA CATTC TAAGC ATTGG AGCAC AGACT GACTT
2001    CCTTT CTGTC TTCTT CTCTG GATAT ACCTT CAAAC ACAAA ATGGT CTATG
2051    AAGAC ACACT CACCC TATTC CCATT CTCAG GAGAA ACTGT CTTCA TGTCG
2101    ATGGA AAACC CAGGT CTATG GATTC TGGGG TGCCA CAACT CAGAC TTTCG
2151    GAACA GAGGC ATGAC CGCCT TACTG AAGGT TTCTA GTTGT GACAA GAACA
2201    CTGGT GATTA TTACG AGGAC AGTTA TGAAG ATATT TCAGC ATACT TGCTG
2251    AGTAA AAACA ATGCC ATTGA ACCAA GAAGC TTCTC TCAAA ACGGC GCGCC
2301    AACAT CAGAG AGCGC CACCC CTGAA AGTGG TCCCG GGAGC GAGCC AGCCA
2351    CATCT GGGTC GGAAA CGCCA GGCAC AAGTG AGTCT GCAAC TCCCG AGTCC
2401    GGACC TGGCT CCGAG CCTGC CACTA GCGGC TCCGA GACTC CGGGA ACTTC
2451    CGAGA GCGCT ACACC AGAAA GCGGA CCCGG AACCA GTACC GAACC TAGCG
2501    AGGGC TCTGC TCCGG GCAGC CCAGC CGGCT CTCCT ACATC CACGG AGGAG
2551    GGCAC TTCCG AATCC GCCAC CCCGG AGTCA GGGCC AGGAT CTGAA CCCGC
2601    TACCT CAGGC AGTGA GACGC CAGGA ACGAG CGAGT CCGCT ACACC GGAGA
2651    GTGGG CCAGG GAGCC CTGCT GGATC TCCTA CGTCC ACTGA GGAAG GGTCA
2701    CCAGC GGGCT CGCCC ACCAG CACTG AAGAA GGTGC CTCGA GCCCA CCAGT
2751    CTTGA AACGC CATCA AGCTG AAATA ACTCG TACTA CTCTT CAGTC AGATC
2801    AAGAG GAAAT CGATT ATGAT GATAC CATAT CAGTT GAAAT GAAGA AGGAA
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2901    AAAGA AAACA CGACA CTATT TTATT GCTGC AGTGG AGAGG CTCTG GGATT
2951    ATGGG ATGAG TAGCT CCCCA CATGT TCTAA GAAAC AGGGC TCAGA GTGGC
3001    AGTGT CCCTC AGTTC AAGAA AGTTG TTTTC CAGGA ATTTA CTGAT GGCTC
3051    CTTTA CTCAG CCCTT ATACC GTGGA GAACT AAATG AACAT TTGGG ACTCC
3101    TGGGG CCATA TATAA GAGCA GAAGT TGAAG ATAAT ATCAT GGTAA CTTTC
3151    AGAAA TCAGG CCTCT CGTCC CTATT CCTTC TATTC TAGCC TTATT TCTTA
3201    TGAGG AAGAT CAGAG GCAAG GAGCA GAACC TAGAA AAAAC TTTGT CAAGC
3251    CTAAT GAAAC CAAAA CTTAC TTTTG GAAAG TGCAA CATCA TATGG CACCC
3301    ACTAA AGATG AGTTT GACTG CAAAG CCTGG GCTTA TTTCT CTGAT GTTGA
3351    CCTGG AAAAA GATGT GCACT CAGGC CTGAT TGGAC CCCTT CTGGT CTGCC
3401    ACACT AACAC ACTGA ACCCT GCTCA TGGGA GACAA GTGAC AGTAC AGGAA
3451    TTTGC TCTGT TTTTC ACCAT CTTTG ATGAG ACCAA AAGCT GGTAC TTCAC
3501    TGAAA ATATG GAAAG AAACT GCAGG GCTCC CTGCA ATATC CAGAT GGAAG
3551    ATCCC ACTTT TAAAG AGAAT TATCG CTTCC ATGCA ATCAA TGGCT ACATA
3601    ATGGA TACAC TACCT GGCTT AGTAA TGGCT CAGGA TCAAA GGATT CGATG
3651    GTATC TGCTC AGCAT GGGCA GCAAT GAAAA CATCC ATTCT ATTCA TTTCA

3701    GTGGA CATGT GTTCA CTGTA CGAAA AAAAG AGGAG TATAA AATGG CACTG
3751    TACAA TCTCT ATCCA GGTGT TTTTG AGACA GTGGA AATGT TACCA TCCAA
3801    AGCTG GAATT TGGCG GGTGG AATGC CTTAT TGGCG AGCAT CTACA TGCTG
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3901    GGAAT GGCTT CTGGA CACAT TAGAG ATTTT CAGAT TACAG CTTCA GGACA
3951    ATATG GACAG TGGGC CCCAA AGCTG GCCAG ACTTC ATTAT TCCGG ATCAA
4001    TCAAT GCCTG GAGCA CCAAG GAGCC CTTTT CTTGG ATCAA GGTGG ATCTG
4051    TTGGC ACCAA TGATT ATTCA CGGCA TCAAG ACCCA GGGTG CCCGT CAGAA
4101    GTTCT CCAGC CTCTA CATCT CTCAG TTTAT CATCA TGTAT AGTCT TGATG
4151    GGAAG AAGTG GCAGA CTTAT CGAGG AAATT CCACT GGAAC CTTAA TGGTC
4201    TTCTT TGGCA ATGTG GATTC ATCTG GGATA AAACA CAATA TTTTT AACCC
4251    TCCAA TTATT GCTCG ATACA TCCGT TTGCA CCCAA CTCAT TATAG CATTC
4301    GCAGC ACTCT TCGCA TGGAG TTGAT GGGCT GTGAT TTAAA TAGTT GCAGC
4351    ATGCC ATTGG GAATG GAGAG TAAAG CAATA TCAGA TGCAC AGATT ACTGC
4401    TTCAT CCTAC TTTAC CAATA TGTTT GCCAC CTGGT CTCCT TCAAA AGCTC
4451    GACTT CACCT CCAAG GGAGG AGTAA TGCCT GGAGA CCTCA GGTGA ATAAT
4501    CCAAA AGAGT GGCTG CAAGT GGACT TCCAG AAGAC AATGA AAGTC ACAGG
4551    AGTAA CTACT CAGGG AGTAA AATCT CTGCT TACCA GCATG TATGT GAAGG
4601    AGTTC CTCAT CTCCA GCAGT CAAGA TGGCC ATCAG TGGAC TCTCT TTTTT
4651    CAGAA TGGCA AAGTA AAGGT TTTTC AGGGA AATCA AGACT CCTTC ACACC
4701    TGTGG TGAAC TCTCT AGACC CACCG TTACT GACTC GCTAC CTTCG AATTC
4751    ACCCC CAGAG TTGGG TGCAC CAGAT TGCCC TGAGG ATGGA GGTTC TGGGC
4801    TGCGA GGCAC AGGAC CTCTA CGACA AAACT CACAC ATGCC CACCG TGCCC
4851    AGCTC CAGAA CTCCT GGGCG GACCG TCAGT CTTCC TCTTC CCCCC AAAAC
4901    CCAAG GACAC CCTCA TGATC TCCCG GACCC CTGAG GTCAC ATGCG TGGTG
4951    GTGGA CGTGA GCCAC GAAGA CCCTG AGGTC AAGTT CAACT GGTAC GTGGA
5001    CGGCG TGGAG GTGCA TAATG CCAAG ACAAA GCCGC GGGAG GAGCA GTACA
5051    ACAGC ACGTA CCGTG TGGTC AGCGT CCTCA CCGTC CTGCA CCAGG ACTGG
5101    CTGAA TGGCA AGGAG TACAA GTGCA AGGTC TCCAA CAAAG CCCTC CCAGC
5151    CCCCA TCGAG AAAAC CATCT CCAAA GCCAA AGGGC AGCCC CGAGA ACCAC
5201    AGGTG TACAC CCTGC CCCCA TCCCG GGATG AGCTG ACCAA GAACC AGGTC
5251    AGCCT GACCT GCCTG GTCAA AGGCT TCTAT CCCAG CGACA TCGCC GTGGA
5301    GTGGG AGAGC AATGG GCAGC CGGAG AACAA CTACA AGACC ACGCC TCCCG
5351    TGTTG GACTC CGACG GCTCC TTCTT CCTCT ACAGC AAGCT CACCG TGGAC
5401    AAGAG CAGGT GGCAG CAGGG GAACG TCTTC TCATG CTCCG TGATG CATGA
5451    GGCTC TGCAC AACCA CTACA CGCAG AAGAG CCTCT CCCTG TCTCC GGGTA
5501    AATGA
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FVIII 282 protein sequence(SEQ ID NO: 159)

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 51    PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
101    DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
151    GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
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201    GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
251    HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
301    RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
351    EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
401    WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
451    TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
501    DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
551    YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
601    NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
651    HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
701    MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
751    SKNNAIEPRS FSQNGAPTSE SATPESGPGS EPATSGSETP GTSESATPES
801    GPGSEPATSG SETPGTSESA TPESGPGTST EPSEGSAPGS PAGSPTSTEE
851    GTSESATPES GPGSEPATSG SETPGTSESA TPESGPGSPA GSPTSTEEGS
901    PAGSPTSTEE GASSPPVLKR HQAEITRTTL QSDQEEIDYD DTISVEMKKE

951    DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLRNRAQSG
1001   SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
1051   RNQASRPYSF YSSLISYEED QRQGAEPRKN FVKPNETKTY FWKVQHHMAP
1101   TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
1151   FALFFTIFDE TKSWYFTENM ERNCRAPCNI QMEDPTFKEN YRFHAINGYI
1201   MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
1251   YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
1301   GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK EPFSWIKVDL
1351   LAPMIIHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
1401   FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
1451   MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
1501   PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISSS QDGHQWTLFF
1551   QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH QIALRMEVLG
1601   CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
1651   VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
1701   LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
1751   SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
1801   KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*
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FVIII 283 nucleotide sequence (FVIII 169 with IHH triple mutation) (SEQ ID NO: 160)

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1      ATGCA AATAG AGCTC TCCAC CTGCT TCTTT CTGTG CCTTT TGCGA TTCTG
51     CTTTA GTGCC ACCAG AAGAT ACTAC CTGGG TGCAG TGGAA CTGTC ATGGG
101    ACTAT ATGCA AAGTG ATCTC GGTGA GCTGC CTGTG GACGC AAGAT TTCCT
151    CCTAG AGTGC CAAAA TCTTT TCCAT TCAAC ACCTC AGTCG TGTAC AAAAA
201    GACTC TGTTT GTAGA ATTCA CGGAT CACCT TTTCA ACATC GCTAA GCCAA
251    GGCCA CCCTG GATGG GTCTG CTAGG TCCTA CCATC CAGGC TGAGG TTTAT
301    GATAC AGTGG TCATT ACACT TAAGA ACATG GCTTC CCATC CTGTC AGTCT
351    TCATG CTGTT GGTGT ATCCT ACTGG AAAGC TTCTG AGGGA GCTGA ATATG
401    ATGAT CAGAC CAGTC AAAGG GAGAA AGAAG ATGAT AAAGT CTTCC CTGGT
451    GGAAG CCATA CATAT GTCTG GCAGG TCCTG AAAGA GAATG GTCCA ATGGC
501    CTCTG ACCCA CTGTG CCTTA CCTAC TCATA TCTTT CTCAT GTGGA CCTGG
551    TAAAA GACTT GAATT CAGGC CTCAT TGGAG CCCTA CTAGT ATGTA GAGAA
601    GGGAG TCTGG CCAAG GAAAA GACAC AGACC TTGCA CAAAT TTATA CTACT
651    TTTTG CTGTA TTTGA TGAAG GGAAA AGTTG GCACT CAGAA ACAAA GAACT
701    CCTTG ATGCA GGATA GGGAT GCTGC ATCTG CTCGG GCCTG GCCTA AAATG
751    CACAC AGTCA ATGGT TATGT AAACA GGTCT CTGCC AGGTC TGATT GGATG
801    CCACA GGAAA TCAGT CTATT GGCAT GTGAT TGGAA TGGGC ACCAC TCCTG
851    AAGTG CACTC AATAT TCCTC GAAGG TCACA CATTT CTTGT GAGGA ACCAT
901    CGCCA GGCTA GCTTG GAAAT CTCGC CAATA ACTTT CCTTA CTGCT CAAAC
951    ACTCT TGATG GACCT TGGAC AGTTT CTACT GTTTT GTCAT ATCTC TTCCC
1001   ACCAA CATGA TGGCA TGGAA GCTTA TGTCA AAGTA GACAG CTGTC CAGAG
1051   GAACC CCAAC TACGA ATGAA AAATA ATGAA GAAGC GGAAG ACTAT GATGA
1101   TGATC TTACT GATTC TGAAA TGGAT GTGGT CAGGT TTGAT GATGA CAACT
1151   CTCCT TCCTT TATCC AAATT CGCTC AGTTG CCAAG AAGCA TCCTA AAACT
1201   TGGGT ACATT ACATT GCTGC TGAAG AGGAG GACTG GGACT ATGCT CCCTT
1251   AGTCC TCGCC CCCGA TGACA GAAGT TATAA AAGTC AATAT TTGAA CAATG
1301   GCCCT CAGCG GATTG GTAGG AAGTA CAAAA AAGTC CGATT TATGG CATAC
1351   ACAGA TGAAA CCTTT AAGAC TCGTG AAGCT ATTCA GCATG AATCA GGAAT
1401   CTTGG GACCT TTACT TTATG GGGAA GTTGG AGACA CACTG TTGAT TATAT
1451   TTAAG AATCA AGCAA GCAGA CCATA TAACA TCTAC CCTCA CGGAA TCACT
1501   GATGT CCGTC CTTTG TATTC AAGGA GATTA CCAAA AGGTG TAAAA CATTT
1551   GAAGG ATTTT CCAAT TCTGC CAGGA GAAAT ATTCA AATAT AAATG GACAG
1601   TGACT GTAGA AGATG GGCCA ACTAA ATCAG ATCCT CGGTG CCTGA CCCGC
1651   TATTA CTCTA GTTTC GTTAA TATGG AGAGA GATCT AGCTT CAGGA CTCAT
1701   TGGCC CTCTC CTCAT CTGCT ACAAA GAATC TGTAG ATCAA AGAGG AAACC
1751   AGATA ATGTC AGACA AGAGG AATGT CATCC TGTTT TCTGT ATTTG ATGAG

1801   AACCG AAGCT GGTAC CTCAC AGAGA ATATA CAACG CTTTC TCCCC AATCC
1851   AGCTG GAGTG CAGCT TGAGG ATCCA GAGTT CCAAG CCTCC AACAT CATGC
1901   ACAGC ATCAA TGGCT ATGTT TTTGA TAGTT TGCAG TTGTC AGTTT GTTTG
1951   CATGA GGTGG CATAC TGGTA CATTC TAAGC ATTGG AGCAC AGACT GACTT
2001   CCTTT CTGTC TTCTT CTCTG GATAT ACCTT CAAAC ACAAA ATGGT CTATG
2051   AAGAC ACACT CACCC TATTC CCATT CTCAG GAGAA ACTGT CTTCA TGTCG
2101   ATGGA AAACC CAGGT CTATG GATTC TGGGG TGCCA CAACT CAGAC TTTCG
2151   GAACA GAGGC ATGAC CGCCT TACTG AAGGT TTCTA GTTGT GACAA GAACA
2201   CTGGT GATTA TTACG ACGAC AGTTA TGAAG ATATT TCAGC ATACT TGCTG
2251   AGTAA AAACA ATGCC ATTGA ACCAA GAAGC TTCTC TCAAA ACGGC GCGCC
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2301    AGGTA CCTCA GAGTC TGCTA CCCCC GAGTC AGGGC CAGGA TCAGA GCCAG
2351    CCACC TCCGG GTCTG AGACA CCCGG GACTT CCGAG AGTGC CACCC CTGAG
2401    TCCGG ACCCG GGTCC GAGCC CGCCA CTTCC GGCTC CGAAA CTCCC GGCAC
2451    AAGCG AGAGC GCTAC CCCAG AGTCA GGACC AGGAA CATCT ACAGA GCCCT
2501    CTGAA GGCTC CGCTC CAGGG TCCCC AGCCG GCAGT CCCAC TAGCA CCGAG
2551    GAGGG AACCT CTGAA AGCGC CACAC CCGAA TCAGG GCCAG GGTCT GAGCC
2601    TGCTA CCAGC GGCAG CGAGA CACCA GGCAC CTCTG AGTCC GCCAC ACCAG
2651    AGTCC GGACC CGGAT CTCCC GCTGG GAGCC CCACC TCCAC TGAGG AGGGA
2701    TCTCC TGCTG GCTCT CCAAC ATCTA CTGAG GAAGG TACCT CAACC GAGCC
2751    ATCCG AGGGA TCAGC TCCCG GCACC TCAGA GTCGG CAACC CCGGA GTCTG
2801    GACCC GGAAC TTCCG AAAGT GCCAC ACCAG AGTCC GGTCC CGGGA CTTCA
2851    GAATC AGCAA CACCC GAGTC CGGCC CTGGG TCTGA ACCCG CCACA AGTGG
2901    TAGTG AGACA CCAGG ATCAG AACCT GCTAC CTCAG GGTCA GAGAC ACCCG
2951    GATCT CCGGC AGGCT CACCA ACCTC CACTG AGGAG GGCAC CAGCA CAGAA
3001    CCAAG CGAGG GCTCC GCACC CGGAA CAAGC ACTGA ACCCA GTGAG GGTTC
3051    AGCAC CCGGC TCTGA GCCGG CCACA AGTGG CAGTG AGACA CCCGG CACTT
3101    CAGAG AGTGC CACCC CCGAG AGTGG CCCAG GCACT AGTAC CGAGC CCTCT
3151    GAAGG CAGTG CGCCA GCCTC GAGCC CACCA GTCTT GAAAC GCCAT CAAGC
3201    TGAAA TAACT CGTAC TACTC TTCAG TCAGA TCAAG AGGAA ATCGA TTATG
3251    ATGAT ACCAT ATCAG TTGAA ATGAA GAAGG AAGAT TTTGA CATTT ATGAT
3301    GAGGA TGAAA ATCAG AGCCC CCGCA GCTTT CAAAA GAAAA CACGA CACTA
3351    TTTTA TTGCT GCAGT GGAGA GGCTC TGGGA TTATG GGATG AGTAG CTCCC
3401    CACAT GTTCT AAGAA ACAGG GCTCA GAGTG GCAGT GTCCC TCAGT TCAAG
3451    AAAGT TGTTT TCCAG GAATT TACTG ATGGC TCCTT TACTC AGCCC TTATA
3501    CCGTG GAGAA CTAAA TGAAC ATTTG GGACT CCTGG GGCCA TATAT AAGAG
3551    CAGAA GTTGA AGATA ATATC ATGGT AACTT TCAGA AATCA GGCCT CTCGT
3601    CCCTA TTCCT TCTAT TCTAG CCTTA TTTCT TATGA GGAAG ATCAG AGGCA
3651    AGGAG CAGAA CCTAG AAAAA ACTTT GTCAA GCCTA ATGAA ACCAA AACTT
3701    ACTTT TGGAA AGTGC AACAT CATAT GGCAC CCACT AAAGA TGAGT TTGAC
3751    TGCAA AGCCT GGGCT TATTT CTCTG ATGTT GACCT GGAAA AAGAT GTGCA
3801    CTCAG GCCTG ATTGG ACCCC TTCTG GTCTG CCACA CTAAC ACACT GAACC
3851    CTGCT CATGG GAGAC AAGTG ACAGT ACAGG AATTT GCTCT GTTTT TCACC
3901    ATCTT TGATG AGACC AAAAG CTGGT ACTTC ACTGA AAATA TGGAA AGAAA
3951    CTGCA GGGCT CCCTG CAATA TCCAG ATGGA AGATC CCACT TTTAA AGAGA
4001    ATTAT CGCTT CCATG CAATC AATGG CTACA TAATG GATAC ACTAC CTGGC
4051    TTAGT AATGG CTCAG GATCA AAGGA TTCGA TGGTA TCTGC TCAGC ATGGG
4101    CAGCA ATGAA AACAT CCATT CTATT CATTT CAGTG GACAT GTGTT CACTG
4151    TACGA AAAAA AGAGG AGTAT AAAAT GGCAC TGTAC AATCT CTATC CAGGT
4201    GTTTT TGAGA CAGTG GAAAT GTTAC CATCC AAAGC TGGAA TTTGG CGGGT
4251    GGAAT GCCTT ATTGG CGAGC ATCTA CATGC TGGGA TGAGC ACACT TTTTC
4301    TGGTG TACAG CAATA AGTGT CAGAC TCCCC TGGGA ATGGC TTCTG GACAC
4351    ATTAG AGATT TTCAG ATTAC AGCTT CAGGA CAATA TGGAC AGTGG GCCCC
4401    AAAGC TGGCC AGACT TCATT ATTCC GGATC AATCA ATGCC TGGAG CACCA
4451    AGGAG CCCTT TTCTT GGATC AAGGT GGATC TGTTG GCACC AATGA TTATT
4501    CACGG CATCA AGACC CAGGG TGCCC GTCAG AAGTT CTCCA GCCTC TACAT
4551    CTCTC AGTTT ATCAT CATGT ATAGT CTTGA TGGGA AGAAG TGGCA GACTT
4601    ATCGA GGAAA TTCCA CTGGA ACCTT AATGG TCTTC TTTGG CAATG TGGAT
4651    TCATC TGGGA TAAAA CACAA TATTT TTAAC CCTCC AATTA TTGCT CGATA
4701    CATCC GTTTG CACCC AACTC ATTAT AGCAT TCGCA GCACT CTTCG CATGG

4751    AGTTG ATGGG CTGTG ATTTA AATAG TTGCA GCATG CCATT GGGAA TGGAG
4801    AGTAA AGCAA TATCA GATGC ACAGA TTACT GCTTC ATCCT ACTTT ACCAA
4851    TATGT TTGCC ACCTG GTCTC CTTCA AAAGC TCGAC TTCAC CTCCA AGGGA
4901    GGAGT AATGC CTGGA GACCT CAGGT GAATA ATCCA AAAGA GTGGC TGCAA
4951    GTGGA CTTCC AGAAG ACAAT GAAAG TCACA GGAGT AACTA CTCAG GGAGT
5001    AAAAT CTCTG CTTAC CAGCA TGTAT GTGAA GGAGT TCCTC ATCTC CAGCA
5051    GTCAA GATGG CCATC AGTGG ACTCT CTTTT TTCAG AATGG CAAAG TAAAG
5101    GTTTT TCAGG GAAAT CAAGA CTCCT TCACA CCTGT GGTGA ACTCT CTAGA
5151    CCCAC CGTTA CTGAC TCGCT ACCTT CGAAT TCACC CCCAG AGTTG GGTGC
5201    ACCAG ATTGC CCTGA GGATG GAGGT TCTGG GCTGC GAGGC ACAGG ACCTC
5251    TACGA CAAAA CTCAC ACATG CCCAC CGTGC CCAGC TCCAG AACTC CTGGG
5301    CGGAC CGTCA GTCTT CCTCT TCCCC CCAAA ACCCA AGGAC ACCCT CATGG
5351    CCTCC CGGAC CCCTG AGGTC ACATG CGTGG TGGTG GACGT GAGCC ACGAA
5401    GACCC TGAGG TCAAG TTCAA CTGGT ACGTG GACGG CGTGG AGGTG CATAA
5451    TGCCA AGACA AAGCC GCGGG AGGAG CAGTA CAACA GCACG TACCG TGTGG
5501    TCAGC GTCCT CACCG TCCTG GCCCA GGACT GGCTG AATGG CAAGG AGTAC
5551    AAGTG CAAGG TCTCC AACAA AGCCC TCCCA GCCCC CATCG AGAAA ACCAT
5601    CTCCA AAGCC AAAGG GCAGC CCCGA GAACC ACAGG TGTAC ACCCT GCCCC
5651    CATCC CGGGA TGAGC TGACC AAGAA CCAGG TCAGC CTGAC CTGCC TGGTC
5701    AAAGG CTTCT ATCCC AGCGA CATCG CCGTG GAGTG GGAGA GCAAT GGGCA
5751    GCCGG AGAAC AACTA CAAGA CCACG CCTCC CGTGT TGGAC TCCGA CGGCT
5801    CCTTC TTCCT CTACA GCAAG CTCAC CGTGG ACAAG AGCAG GTGGC AGCAG
5851    GGGAA CGTCT TCTCA TGCTC CGTGA TGCAT GAGGC TCTGC ACAAC GCCTA
5901    CACGC AGAAG AGCCT CTCCC TGTCT CCGGG TAAAT GA
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FVIII 283 protein sequence (FVIII 169 with IHH triple mutation) (SEQ ID NO: 161)

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   1    MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP
  51    PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101    DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 151    GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201    GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251    HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301    RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351    EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401    WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
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 401    WVHTIAALEE DWDYAFEVLA PDDKSYRSQY LNNGPQRIGR KYKKVRFMAY
 451    TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 501    DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551    YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601    NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651    HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 701    MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 751    SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSESATPE
 801    SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
 851    EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP AGSPTSTEEG
 901    SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
 951    ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
1001    PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPGTSTEPS
1051    EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
1101    EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVPQFK
1151    KVVFQEFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
1201    PYSFYSSLIS YEEDQRQGAE PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
1251    CKAWAYFSDV DLEKDVHSGL IGPLLVCHTN TLNPAHGRQV TVQEFALFFT
1301    IFDETKSWYF TENMERNCRA PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
1351    LVMAQDQRIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG

1401    VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
1451    IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
1501    HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
1551    SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
1601    SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
1651    VDFQKTMKVT GVTTQGVKSL LTSMYVKEFL ISSSQDGHQW TLFFQNGKVK
1701    VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
1751    YDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMASRTPEV TCVVVDVSHE
1801    DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL AQDWLNGKEY
1851    KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV
1901    KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
1951    GNVFSCSVMH EALHNAYTQK SLSLSPGK*
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pSYNFVIII 010 nucleotide sequence-(Dual chain FVIIIFc) (SEQ ID NO: 162)

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   1    ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT TGCGATTCTG
  51    CTTTAGTGCC ACCAGAAGAT ACTACCTGGG TGCAGTGGAA CTGTCATGGG
 101    ACTATATGCA AAGTGATCTC GGTGAGCTGC CTGTGGACGC AAGATTTCCT
 151    CCTAGAGTGC CAAAATCTTT TCCATTCAAC ACCTCAGTCG TGTACAAAAA
 201    GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC GCTAAGCCAA
 251    GGCCACCCTG GATGGGTCTG CTAGGTCCTA CCATCCAGGC TGAGGTTTAT
 301    GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTCAGTCT
 351    TCATGCTGTT GGTGTATCCT ACTGGAAAGC TTCTGAGGGA GCTGAATATG
 401    ATGATCAGAC CAGTCAAAGG GAGAAAGAAG ATGATAAAGT CTTCCCTGGT
 451    GGAAGCCATA CATATGTCTG GCAGGTCCTG AAAGAGAATG GTCCAATGGC
 501    CTCTGACCCA CTGTGCCTTA CCTACTCATA TCTTTCTCAT GTGGACCTGG
 551    TAAAAGACTT GAATTCAGGC CTCATTGGAG CCCTACTAGT ATGTAGAGAA
 601    GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT
 651    TTTTGCTGTA TTTGATGAAG GGAAAAGTTG GCACTCAGAA ACAAAGAACT
 701    CCTTGATGCA GGATAGGGAT GCTGCATCTG CTCGGGCCTG GCCTAAAATG
 751    CACACAGTCA ATGGTTATGT AAACAGGTCT CTGCCAGGTC TGATTGGATG
 801    CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAATGGGC ACCACTCCTG
 851    AAGTGCACTC AATATTCCTC GAAGGTCACA CATTTCTTGT GAGGAACCAT
 901    CGCCAGGCGT CCTTGGAAAT CTCGCCAATA ACTTTCCTTA CTGCTCAAAC
 951    ACTCTTGATG GACCTTGGAC AGTTTCTACT GTTTTGTCAT ATCTCTTCCC
1001    ACCAACATGA TGGCATGGAA GCTTATGTCA AAGTAGACAG CTGTCCAGAG
1051    GAACCCCAAC TACGAATGAA AAATAATGAA GAAGCGGAAG ACTATGATGA
1101    TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT GATGACAACT
1151    CTCCTTCCTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA TCCTAAAACT
1201    TGGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCTT
1251    AGTCCTCGCC CCCGATGACA GAAGTTATAA AAGTCAATAT TTGAACAATG
1301    GCCCTCAGCG GATTGGTAGG AAGTACAAAA AAGTCCGATT TATGGCATAC
1351    ACAGATGAAA CCTTTAAGAC TCGTGAAGCT ATTCAGCATG AATCAGGAAT
1401    CTTGGGACCT TTACTTTATG GGGAAGTTGG AGACACACTG TTGATTATAT
1451    TTAAGAATCA AGCAAGCAGA CCATATAACA TCTACCCTCA CGGAATCACT
1501    GATGTCCGTC CTTTGTATTC AAGGAGATTA CCAAAAGGTG TAAAACATTT
1551    GAAGGATTTT CCAATTCTGC CAGGAGAAAT ATTCAAATAT AAATGGACAG
1601    TGACTGTAGA AGATGGGCCA ACTAAATCAG ATCCTCGGTG CCTGACCCGC
1651    TATTACTCTA GTTTCGTTAA TATGGAGAGA GATCTAGCTT CAGGACTCAT
1701    TGGCCCTCTC CTCATCTGCT ACAAAGAATC TGTAGATCAA AGAGGAAACC
1751    AGATAATGTC AGACAAGAGG AATGTCATCC TGTTTTCTGT ATTTGATGAG
1801    AACCGAAGCT GGTACCTCAC AGAGAATATA CAACGCTTTC TCCCCAATCC
1851    AGCTGGAGTG CAGCTTGAGG ATCCAGAGTT CCAAGCCTCC AACATCATGC
1901    ACAGCATCAA TGGCTATGTT TTTGATAGTT TGCAGTTGTC AGTTTGTTTG
1951    CATGAGGTGG CATACTGGTA CATTCTAAGC ATTGGAGCAC AGACTGACTT
2001    CCTTTCTGTC TTCTTCTCTG GATATACCTT CAAACACAAA ATGGTCTATG
2051    AAGACACACT CACCCTATTC CCATTCTCAG GAGAAACTGT CTTCATGTCG
2101    ATGGAAAACC CAGGTCTATG GATTCTGGGG TGCCACAACT CAGACTTTCG
2151    GAACAGAGGC ATGACCGCCT TACTGAAGGT TTCTAGTTGT GACAAGAACA

2201    CTGGTGATTA TTACGAGGAC AGTTATGAAG ATATTTCAGC ATACTTGCTG
2251    AGTAAAAACA ATGCCATTGA ACCAAGAAGC TTCTCTCAAA ACCCACCAGT
2301    CTTGAAACGC CATCAACGGG AAATAACTCG TACTACTCTT CAGTCAGATC
2351    AAGAGGAAAT TGACTATGAT GATACCATAT GACTTCAAAT GAAGAAGGAA
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2351  AAGAGGAAAT TGACTATGAT GATACCATAT CAGTTGAAAT GAAGAAGGAA
2401  GATTTTGACA TTTATGATGA GGATGAAAAT CAGAGCCCCC GCAGCTTTCA
2451  AAAGAAAACA CGACACTATT TTATTGCTGC AGTGGAGAGG CTCTGGGATT
2501  ATGGGATGAG TAGCTCCCCA CATGTTCTAA GAAACAGGGC TCAGAGTGGC
2551  AGTGTCCCTC AGTTCAAGAA AGTTGTTTTC CAGGAATTTA CTGATGGCTC
2601  CTTTACTCAG CCCTTATACC GTGGAGAACT AAATGAACAT TTGGGACTCC
2651  TGGGGCCATA TATAAGAGCA GAAGTTGAAG ATAATATCAT GGTAACTTTC
2701  AGAAATCAGG CCTCTCGTCC CTATTCCTTC TATTCTAGCC TTATTTCTTA
2751  TGAGGAAGAT CAGAGGCAAG GAGCAGAACC TAGAAAAAAC TTTGTCAAGC
2801  CTAATGAAAC CAAAACTTAC TTTTGGAAAG TGCAACATCA TATGGCACCC
2851  ACTAAAGATG AGTTTGACTG CAAAGCCTGG GCTTATTTCT CTGATGTTGA
2901  CCTGGAAAAA GATGTGCACT CAGGCCTGAT TGGACCCCTT CTGGTCTGCC
2951  ACACTAACAC ACTGAACCCT GCTCATGGGA GACAAGTGAC AGTACAGGAA
3001  TTTGCTCTGT TTTTCACCAT CTTTGATGAG ACCAAAAGCT GGTACTTCAC
3051  TGAAAATATG GAAAGAAACT GCAGGGCTCC CTGCAATATC CAGATGGAAG
3101  ATCCCACTTT TAAAGAGAAT TATCGCTTCC ATGCAATCAA TGGCTACATA
3151  ATGGATACAC TACCTGGCTT AGTAATGGCT CAGGATCAAA GGATTCGATG
3201  GTATCTGCTC AGCATGGGCA GCAATGAAAA CATCCATTCT ATTCATTTCA
3251  GTGGACATGT GTTCACTGTA CGAAAAAAAG AGGAGTATAA AATGGCACTG
3301  TACAATCTCT ATCCAGGTGT TTTTGAGACA GTGGAAATGT TACCATCCAA
3351  AGCTGGAATT TGGCGGGTGG AATGCCTTAT TGGCGAGCAT CTACATGCTG
3401  GGATGAGCAC ACTTTTTCTG GTGTACAGCA ATAAGTGTCA GACTCCCCTG
3451  GGAATGGCTT CTGGACACAT TAGAGATTTT CAGATTACAG CTTCAGGACA
3501  ATATGGACAG TGGGCCCCAA AGCTGGCCAG ACTTCATTAT TCCGGATCAA
3551  TCAATGCCTG GAGCACCAAG GAGCCCTTTT CTTGGATCAA GGTGGATCTG
3601  TTGGCACCAA TGATTATTCA CGGCATCAAG ACCCAGGGTG CCCGTCAGAA
3651  GTTCTCCAGC CTCTACATCT CTCAGTTTAT CATCATGTAT AGTCTTGATG
3701  GGAAGAAGTG GCAGACTTAT CGAGGAAATT CCACTGGAAC CTTAATGGTC
3751  TTCTTTGGCA ATGTGGATTC ATCTGGGATA AAACACAATA TTTTTAACCC
3801  TCCAATTATT GCTCGATACA TCCGTTTGCA CCCAACTCAT TATAGCATTC
3851  GCAGCACTCT TCGCATGGAG TTGATGGGCT GTGATTTAAA TAGTTGCAGC
3901  ATGCCATTGG GAATGGAGAG TAAAGCAATA TCAGATGCAC AGATTACTGC
3951  TTCATCCTAC TTTACCAATA TGTTTGCCAC CTGGTCTCCT TCAAAAGCTC
4001  GACTTCACCT CCAAGGGAGG AGTAATGCCT GGAGACCTCA GGTGAATAAT
4051  CCAAAAGAGT GGCTGCAAGT GGACTTCCAG AAGACAATGA AAGTCACAGG
4101  AGTAACTACT CAGGGAGTAA AATCTCTGCT TACCAGCATG TATGTGAAGG
4151  AGTTCCTCAT CTCCAGCAGT CAAGATGGCC ATCAGTGGAC TCTCTTTTTT
4201  CAGAATGGCA AAGTAAAGGT TTTTCAGGGA AATCAAGACT CCTTCACACC
4251  TGTGGTGAAC TCTCTAGACC CACCGTTACT GACTCGCTAC CTTCGAATTC
4301  ACCCCCAGAG TTGGGTGCAC CAGATTGCCC TGAGGATGGA GGTTCTGGGC
4351  TGCGAGGCAC AGGACCTCTA CGACAAAACT CACACATGCC CACCGTGCCC
4401  AGCTCCAGAA CTCCTGGGCG GACCGTCAGT CTTCCTCTTC CCCCCAAAAC
4451  CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTCAC ATGCGTGGTG
4501  GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTTCAACT GGTACGTGGA
4551  CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
4601  ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG
4651  CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC
4701  CCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACCAC
4751  AGGTGTACAC CCTGCCCCCA TCCCGGGATG AGCTGACCAA GAACCAGGTC
4801  AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA TCGCCGTGGA
4851  GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCCG
4901  TGTTGGACTC CGACGGCTCC TTCTTCCTCT ACAGCAAGCT CACCGTGGAC
4951  AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA
5001  GGCTCTGCAC AACCACTACA CGCAGAAGAG CCTCTCCCTG TCTCCGGGTA
5051  AATGA
```

pSYNFVIII 010 protein sequence-(Dual chain FVIIIFc) (SEQ ID NO: 163)

```
   1  MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP
  51  PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101  DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 151  GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201  GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251  HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301  RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351  EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401  WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451  TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 501  DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551  YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601  NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651  HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 701  MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 751  SKNNAIEPRS FSQNPPVLKR HQREITRTTL QSDQEEIDYD DTISVEMKKE
 801  DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLRNRAQSG
 851  SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
 901  RNQASRPYSF YSSLISYEED QRQGAEPRKN FVKPNETKTY FWKVQHHMAP
 951  TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
1001  FALFFTIFDE TKSWYFTENM ERNCRAPCNI QMEDPTFKEN YRFHAINGYI
1051  MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
1101  YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
1151  GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK EPFSWIKVDL
1201  LAPMIIHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
1251  FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
1301  MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
1351  PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISSS QDGHQWTLFF
```

```
1351    PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISSS QDGHQWTLFF
1401    QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH QIALRMEVLG
1451    CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
1501    VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
1551    LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
1601    SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
1651    KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*
```

FVIII 195 protein sequence (dual chain FVIIIFc with two 144 AE XTENs at amino acid 1656 and 1900) (SEQ ID NO: 73)

```
   1    MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP
  51    PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101    DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 151    GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201    GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251    HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301    RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351    EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401    WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451    TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 501    DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551    YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601    NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651    HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 701    MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 751    SKNNAIEPRS FSQNPPVLKR HQREITRTTL QGAPGTPGSG TASSSPGASP
 801    GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSPSAST GTGPGTPGSG
 851    TASSSPGASP GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSTPSGA
 901    TGSPGSSTPS GATGSPGASP GTSSTGSPAS SSDQEEIDYD DTISVEMKKE

 951    DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLRNRAQSG
1001    SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
1051    RNQASRPYSF YSSLISYEED QRQGAEPRKN FVKPNETKTY FWKVQHHMAP
1101    TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
1151    FALFFTIFDE TKSWYFTENM ERNCRGAPTS ESATPESGPG SEPATSGSET
1201    PGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG
1251    TSESATPESG PGSPAGSPTS TEEGSPAGSP TSTEEGSPAG SPTSTEEGTS
1301    ESATPESGPG TSTEPSEGSA PGASSAPCNI QMEDPTFKEN YRFHAINGYI
1351    MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
1401    YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
1451    GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK EPFSWIKVDL
1501    LAPMIIHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
1551    FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
1601    MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
1651    PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISSS QDGHQWTLFF
1701    QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH QIALRMEVLG
1751    CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
1801    VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
1851    LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
1901    SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
1951    KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*
```

pSYN-FVIII-173 mature Protein sequencing (SEQ ID NO: 72):

```
   1  ATRRYYLGAV ELSWDYMQSD LGELPVDARF PPRVPKSFPF NTSVVYKKTL
  51  FVEFTDHLFN IAKPRPPWMG LLGPTIQAEV YDTVVITLKN MASHPVSLHA
 101  VGVSYWKASE GAEYDDQTSQ REKEDDKVFP GGSHTYVWQV LKENGPMASD
 151  PLCLTYSYLS HVDLVKDLNS GLIGALLVCR EGSLAKEKTQ TLHKFILLFA
 201  VFDEGKSWHS ETKNSLMQDR DAASARAWPK MHTVNGYVNR SLPGLIGCHR
 251  KSVYWHVIGM GTTPEVHSIF LEGHTFLVRN HRQASLEISP ITFLTAQTLL
 301  MDLGQFLLFC HISSHQHDGM EAYVKVDSCP EEPQLRMKNN EEAEDYDDDL
 351  TDSEMDVVRF DDDNSPSFIQ IRSVAKKHPK TWVHYIAAEE EDWDYAPLVL
 401  APDDRSYKSQ YLNNGPQRIG RKYKKVRFMA YTDETFKTRE AIQHESGILG
 451  PLLYGEVGDT LLIIFKNQAS RPYNIYPHGI TDVRPLYSRR LPKGVKHLKD
 501  FPILPGEIFK YKWTVTVEDG PTKSDPRCLT RYYSSFVNME RDLASGLIGP
 551  LLICYKESVD QRGNQIMSDK RNVILFSVFD ENRSWYLTEN IQRFLPNPAG
 601  VQLEDPEFQA SNIMHSINGY VFDSLQLSVC LHEVAYWYIL SIGAQTDFLS
 651  VFFSGYTFKH KMVYEDTLTL FPFSGETVFM SMENPGLWIL GCHNSDFRNR
 701  GMTALLKVSS CDKNTGDYYE DSYEDISAYL LSKNNAIEPR SFSQNGAPGT
 751  SESATPESGP GSEPATSGSE TPGTSESATP ESGPGSEPAT SGSETPGTSE
 801  SATPESGPGT STEPSEGSAP GSPAGSPTST EEGTSESATP ESGPGSEPAT
 851  SGSETPGTSE SATPESGPGS PAGSPTSTEE GSPAGSPTST EEGTSTEPSE
 901  GSAPGTSESA TPESGPGTSE SATPESGPGT SESATPESGP GSEPATSGSE
 951  TPGSEPATSG SETPGSPAGS PTSTEEGTST EPSEGSAPGT STEPSEGSAP
1001  GSEPATSGSE TPGTSESATP ESGPGTSTEP SEGSAPASSP PVLKRHQREI
1051  TRTTLQSDQE EIDYDDTISV EMKKEDFDIY DEDENQSPRS FQKKTRHYFI
1101  AAVERLWDYG MSSSPHVLRN RAQSGSVPQF KKVVFQEFTD GSFTQPLYRG
1151  ELNEHLGLLG PYIRAEVEDN IMVTFRNQAS RPYSFYSSLI SYEEDQRQGA
1201  EPRKNFVKPN ETKTYFWKVQ HHMAPTKDEF DCKAWAYFSD VDLEKDVHSG
1251  LIGPLLVCHT NTLNPAHGRQ VTVQEFALFF TIFDETKSWY FTENMERNCR
1301  APCNIQMEDP TFKENYRFHA INGYIMDTLP GLVMAQDQRI RWYLLSMGSN
1351  ENIHSIHFSG HVFTVRKKEE YKMALYNLYP GVFETVEMLP SKAGIWRVEC
1401  LIGEHLHAGM STLFLVYSNK CQTPLGMASG HIRDFQITAS GQYGQWAPKL
1451  ARLHYSGSIN AWSTKEPFSW IKVDLLAPMI IHGIKTQGAR QKFSSLYISQ
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1501  FIIMYSLDGK KWQTYRGNST GTLMVFFGNV DSSGIKHNIF NPPIIARYIR
1551  LHPTHYSIRS TLRMELMGCD LNSCSMPLGM ESKAISDAQI TASSYFTNMF
1601  ATWSPSKARL HLQGRSNAWR PQVNNPKEWL QVDFQKTMKV TGVTTQGVKS
1651  LLTSMYVKEF LISSSQDGHQ WTLFFQNGKV KVFQGNQDSF TPVVNSLDPP

1701  LLTRYLRIHP QSWVHQIALR MEVLGCEAQD LYDKTHTCPP CPAPELLGGP
1751  SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK
1801  TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK
1851  AKGQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE
1901  NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ
1951  KSLSLSPGK
```

FVIII 196 protein sequence (dual chain FVIIIFc with three 144 AE XTENs at amino acid 26,1656 and 1900) (SEQ ID NO: 74)

```
   1  MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVGAPGS
  51  SPSASTGTGP GSSPSASTGT GPGASPGTSS TGSPGASPGT SSTGSPGSST
 101  PSGATGSPGS SPSASTGTGP GASPGTSSTG SPGSSPSAST GTGPGTPGSG
 151  TASSSPGSST PSGATGSPGS STPSGATGSP GASPGTSSTG SPASSDARFP
 201  PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 251  DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 301  GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 351  GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 401  HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 451  RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 501  EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 551  WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 601  TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 651  DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 701  YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 751  NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 801  HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 851  MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 901  SKNNAIEPRS FSQNPPVLKR HQREITRTTL QGAPGTPGSG TASSSPGASP
 951  GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSPSAST GTGPGTPGSG
1001  TASSSPGASP GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSTPSGA
1051  TGSPGSSTPS GATGSPGASP GTSSTGSPAS SSDQEEIDYD DTISVEMKKE
1101  DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLRNRAQSG
1151  SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
1201  RNQASRPYSF YSSLISYEED QRQGAEPRKN FVKPNETKTY FWKVQHHMAP
1251  TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
1301  FALFFTIFDE TKSWYFTENM ERNCRGAPTS ESATPESGPG SEPATSGSET
1351  PGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG
1401  TSESATPESG PGSPAGSPTS TEEGSPAGSP TSTEEGSPAG SPTSTEEGTS
1451  ESATPESGPG TSTEPSEGSA PGASSAPCNI QMEDPTFKEN YRFHAINGYI
1501  MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
1551  YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
1601  GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK EPFSWIKVDL
1651  LAPMIIHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
1701  FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
1751  MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
1801  PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISSS QDGHQWTLFF
1851  QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH QIALRMEVLG
1901  CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
1951  VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
2001  LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
2051  SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
2101  KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*
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FVIII 199 protein sequence (single chain FVIIIFc with three 144 AE XTENs at amino acid 1656 and 1900) (SEQ ID NO: 75)

```
   1  MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP
  51  PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101  DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 151  GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201  GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251  HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301  RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351  EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401  WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451  TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 501  DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551  YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601  NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651  HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 701  MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 751  SKNNAIEPRS FSQNPPVLKR HQAEITRTTL QGAPGTPGSG TASSSPGASP
 801  GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSPSAST GTGPGTPGSG
 851  TASSSPGASP GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSTPSGA
 901  TGSPGSSTPS GATGSPGASP GTSSTGSPAS SSDQEEIDYD DTISVEMKKE
 951  DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLRNRAQSG
1001  SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
```

```
1051    RNQASRPYSF YSSLISYEED QRQGAEPRKN FVKPNETKTY FWKVQHHMAP
1101    TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
1151    FALFFTIFDE TKSWYFTENM ERNCRGAPTS ESATPESGPG SEPATSGSET
1201    PGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG
1251    TSESATPESG PGSPAGSPTS TEEGSPAGSP TSTEEGSPAG SPTSTEEGTS
1301    ESATPESGPG TSTEPSEGSA PGASSAPCNI QMEDPTFKEN YRFHAINGYI
1351    MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
1401    YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
1451    GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK EPFSWIKVDL
1501    LAPMIIHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
1551    FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
1601    MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
1651    PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISSS QDGHQWTLFF
1701    QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH QIALRMEVLG
1751    CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
1801    VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
1851    LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
1901    SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
1951    KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*
```

FVIII 201 protein sequence (single chain FVIIIFc with three 144 AE XTENs at amino acid 26,1656 &1900) (SEQ ID NO: 76)

```
   1    MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVGAPGS
  51    SPSASTGTGP GSSPSASTGT GPGASPGTSS TGSPGASPGT SSTGSPGSST
 101    PSGATGSPGS SPSASTGTGP GASPGTSSTG SPGSSPSAST GTGPGTPGSG
 151    TASSSPGSST PSGATGSPGS STPSGATGSP GASPGTSSTG SPASSDARFP
 201    PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 251    DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 301    GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 351    GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 401    HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 451    RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 501    EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 551    WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 601    TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 651    DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR

 701     YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 751     NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 801     HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 851     MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 901     SKNNAIEPRS FSQNPPVLKR HQAEITRTTL QGAPGTPGSG TASSSPGASP
 951     GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSPSAST GTGPGTPGSG
1001     TASSSPGASP GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSTPSGA
1051     TGSPGSSTPS GATGSPGASP GTSSTGSPAS SSDQEEIDYD DTISVEMKKE
1101     DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLRNRAQSG
1151     SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
1201     RNQASRPYSF YSSLISYEED QRQGAEPRKN FVKPNETKTY FWKVQHHMAP
1251     TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
1301     FALFFTIFDE TKSWYFTENM ERNCRGAPTS ESATPESGPG SEPATSGSET
1351     PGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG
1401     TSESATPESG PGSPAGSPTS TEEGSPAGSP TSTEEGSPAG SPTSTEEGTS
1451     ESATPESGPG TSTEPSEGSA PGASSAPCNI QMEDPTFKEN YRFHAINGYI
1501     MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
1551     YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
1601     GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK EPFSWIKVDL
1651     LAPMIIHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
1701     FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
1751     MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
1801     PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISSS QDGHQWTLFF
1851     QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH QIALRMEVLG
1901     CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
1951     VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
2001     LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
2051     SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
2101     KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*
```

FVIII 203 protein sequence (single chain FVIIIFc with two AE XTENs; one 288AE XTEN in B-domain and one 144 AE XTEN at amino acid 1900) (SEQ ID NO: 77)

```
   1    MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP
  51    PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101    DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 151    GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201    GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251    HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301    RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351    EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401    WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451    TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 501    DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551    YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601    NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
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 651    HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 701    MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 751    SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSESATPE
 801    SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
 851    EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP AGSPTSTEEG
 901    SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
 951    ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
1001    PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPGTSTEPS
1051    EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
1101    EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVPQFK
1151    KVVFQEFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
1201    PYSFYSSLIS YEEDQRQGAE PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
1251    CKAWAYFSDV DLEKDVHSGL IGPLLVCHTN TLNPAHGRQV TVQEFALFFT

1301    IFDETKSWYF TENMERNCRG APTSESATPE SGPGSEPATS GSETPGTSES
1351    ATPESGPGSE PATSGSETPG TSESATPESG PGTSTEPSEG SAPGTSESAT
1401    PESGPGSPAG SPTSTEEGSP AGSPTSTEEG SPAGSPTSTE EGTSESATPE
1451    SGPGTSTEPS EGSAPGASSA PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
1501    LVMAQDQRIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
1551    VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
1601    IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
1651    HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
1701    SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
1751    SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
1801    VDFQKTMKVT GVTTQGVKSL LTSMYVKEFL ISSSQDGHQW TLFFQNGKVK
1851    VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
1901    YDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
1951    DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
2001    KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV
2051    KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
2101    GNVFSCSVMH EALHNHYTQK SLSLSPGK*
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FVIII 204 protein sequence (single chain FVIIIFc with two AE XTENs; one 288AE XTEN in B-domain and one 144 AE XTEN at amino acid 403) (SEQ ID NO: 78)

```
   1    MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP
  51    PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101    DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 151    GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201    GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251    HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301    RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351    EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401    WVHYIAAEEE DWDYAPLVLA PDGAPTSTEP SEGSAPGSPA GSPTSTEEGT
 451    STEPSEGSAP GTSTEPSEGS APGTSESATP ESGPGTSTEP SEGSAPGTSE
 501    SATPESGPGS EPATSGSETP GTSTEPSEGS APGTSTEPSE GSAPGTSESA
 551    TPESGPGTSE SATPESGPGA SSDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 601    TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 651    DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 701    YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 751    NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 801    HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 851    MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 901    SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSESATPE
 951    SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
1001    EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP AGSPTSTEEG
1051    SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
1101    ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
1151    PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPGTSTEPS
1201    EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
1251    EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVPQFK
1301    KVVFQEFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
1351    PYSFYSSLIS YEEDQRQGAE PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
1401    CKAWAYFSDV DLEKDVHSGL IGPLLVCHTN TLNPAHGRQV TVQEFALFFT
1451    IFDETKSWYF TENMERNCRA PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
1501    LVMAQDQRIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
1551    VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
1601    IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
1651    HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
1701    SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
1751    SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
1801    VDFQKTMKVT GVTTQGVKSL LTSMYVKEFL ISSSQDGHQW TLFFQNGKVK

1851    VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
1901    YDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
1951    DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
2001    KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV
2051    KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
2101    GNVFSCSVMH EALHNHYTQK SLSLSPGK*
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FVIII 205 protein sequence (single chain FVIIIFc with two AE XTENs; one 288AE XTEN in B-domain and

one 144 AE XTEN at amino acid 18) (SEQ ID NO: 79)

```
   1  MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQGAP TSESATPESG
  51  PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG
 101  TSTEPSEGSA PGSPAGSPTS TEEGTSESAT PESGPGSEPA TSGSETPGTS
 151  ESATPESGPG SPAGSPTSTE EGSPAGSPTS TEEGASSSDL GELPVDARFP
 201  PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 251  DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 301  GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 351  GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 401  HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 451  RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 501  EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 551  WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 601  TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 651  DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 701  YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 751  NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 801  HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 851  MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 901  SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSESATPE
 951  SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
1001  EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP AGSPTSTEEG
1051  SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
1101  ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
1151  PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPGTSTEPS
1201  EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
1251  EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVPQFK
1301  KVVFQEFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
1351  PYSFYSSLIS YEEDQRQGAE PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
1401  CKAWAYFSDV DLEKDVHSGL IGPLLVCHTN TLNPAHGRQV TVQEFALFFT
1451  IFDETKSWYF TENMERNCRA PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
1501  LVMAQDQRIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
1551  VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
1601  IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
1651  HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
1701  SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
1751  SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
1801  VDFQKTMKVT GVTTQGVKSL LTSMYVKEFL ISSSQDGHQW TLFFQNGKVK
1851  VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
1901  YDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
1951  DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
2001  KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV
2051  KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
2101  GNVFSCSVMH EALHNHYTQK SLSLSPGK*
```

pSYN FVIII 266 protein sequence (FVIII Fc with 42 AE-XTEN at amino acid 18 and 288 AE XTEN in B-domain) SEQ ID NO: 80)

```
   1  MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQGAP GSPAGSPTST
  51  EEGTSESATP ESGPGSEPAT SGSETPASSS DLGELPVDAR FPPRVPKSFP
 101  FNTSVVYKKT LFVEFTDHLF NIAKPRPPWM GLLGPTIQAE VYDTVVITLK
 151  NMASHPVSLH AVGVSYWKAS EGAEYDDQTS QREKEDDKVF PGGSHTYVWQ
 201  VLKENGPMAS DPLCLTYSYL SHVDLVKDLN SGLIGALLVC REGSLAKEKT
 251  QTLHKFILLF AVFDEGKSWH SETKNSLMQD RDAASARAWP KMHTVNGYVN
 301  RSLPGLIGCH RKSVYWHVIG MGTTPEVHSI FLEGHTFLVR NHRQASLEIS
 351  PITFLTAQTL LMDLGQFLLF CHISSHQHDG MEAYVKVDSC PEEPQLRMKN
 401  NEEAEDYDDD LTDSEMDVVR FDDDNSPSFI QIRSVAKKHP KTWVHYIAAE
 451  EEDWDYAPLV LAPDDRSYKS QYLNNGPQRI GRKYKKVRFM AYTDETFKTR
 501  EAIQHESGIL GPLLYGEVGD TLLIIFKNQA SRPYNIYPHG ITDVRPLYSR
 551  RLPKGVKHLK DFPILPGEIF KYKWTVTVED GPTKSDPRCL TRYYSSFVNM
 601  ERDLASGLIG PLLICYKESV DQRGNQIMSD KRNVILFSVF DENRSWYLTE
 651  NIQRFLPNPA GVQLEDPEFQ ASNIMHSING YVFDSLQLSV CLHEVAYWYI
 701  LSIGAQTDFL SVFFSGYTFK HKMVYEDTLT LFPFSGETVF MSMENPGLWI
 751  LGCHNSDFRN RGMTALLKVS SCDKNTGDYY EDSYEDISAY LLSKNNAIEP
 801  RSFSQNGAPG TSESATPESG PGSEPATSGS ETPGTSESAT PESGPGSEPA
 851  TSGSETPGTS ESATPESGPG TSTEPSEGSA PGSPAGSPTS TEEGTSESAT
 901  PESGPGSEPA TSGSETPGTS ESATPESGPG SPAGSPTSTE EGSPAGSPTS
 951  TEEGTSTEPS EGSAPGTSES ATPESGPGTS ESATPESGPG TSESATPESG
1001  PGSEPATSGS ETPGSEPATS GSETPGSPAG SPTSTEEGTS TEPSEGSAPG
1051  TSTEPSEGSA PGSEPATSGS ETPGTSESAT PESGPGTSTE PSEGSAPASS
1101  PPVLKRHQAE ITRTTLQSDQ EEIDYDDTIS VEMKKEDFDI YDEDENQSPR
1151  SFQKKTRHYF IAAVERLWDY GMSSSPHVLR NRAQSGSVPQ FKKVVFQEFT
1201  DGSFTQPLYR GELNEHLGLL GPYIRAEVED NIMVTFRNQA SRPYSFYSSL
1251  ISYEEDQRQG AEPRKNFVKP NETKTYFWKV QHHMAPTKDE FDCKAWAYFS
1301  DVDLEKDVHS GLIGPLLVCH TNTLNPAHGR QVTVQEFALF FTIFDETKSW
1351  YFTENMERNC RAPCNIQMED PTFKENYRFH AINGYIMDTL PGLVMAQDQR
1401  IRWYLLSMGS NENIHSIHFS GHVFTVRKKE EYKMALYNLY PGVFETVEML
1451  PSKAGIWRVE CLIGEHLHAG MSTLFLVYSN KCQTPLGMAS GHIRDFQITA
1501  SGQYGQWAPK LARLHYSGSI NAWSTKEPFS WIKVDLLAPM IIHGIKTQGA
1551  RQKFSSLYIS QFIIMYSLDG KKWQTYRGNS TGTLMVFFGN VDSSGIKHNI
1601  FNPPIIARYI RLHPTHYSIR STLRMELMGC DLNSCSMPLG MESKAISDAQ
1651  ITASSYFTNM FATWSPSKAR LHLQGRSNAW RPQVNNPKEW LQVDFQKTMK
1701  VTGVTTQGVK SLLTSMYVKE FLISSSQDGH QWTLFFQNGK VKVFQGNQDS
1751  FTPVVNSLDP PLLTRYLRIH PQSWVHQIAL RMEVLGCEAQ DLYDKTHTCP
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1801    PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW
1851    YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA
1901    LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI
1951    AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV
2001    MHEALHNHYT QKSLSLSPGK *
```

pSYN FVIII 267 protein sequence (FVIII Fc with 72 AE-XTEN at amino acid 18 and 288 AE XTEN in B-domain) SEQ ID NO: 81)

```
   1    MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQGAP TSESATPESG
  51    PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG
 101    TSTEPSEGSA PGASSSDLGE LPVDARFPPR VPKSFPFNTS VVYKKTLFVE
 151    FTDHLFNIAK PRPPWMGLLG PTIQAEVYDT VVITLKNMAS HPVSLHAVGV
 201    SYWKASEGAE YDDQTSQREK EDDKVFPGGS HTYVWQVLKE NGPMASDPLC
 251    LTYSYLSHVD LVKDLNSGLI GALLVCREGS LAKEKTQTLH KFILLFAVFD
 301    EGKSWHSETK NSLMQDRDAA SARAWPKMHT VNGYVNRSLP GLIGCHRKSV
 351    YWHVIGMGTT PEVHSIFLEG HTFLVRNHRQ ASLEISPITF LTAQTLLMDL
 401    GQFLLFCHIS SHQHDGMEAY VKVDSCPEEP QLRMKNNEEA EDYDDDLTDS
 451    EMDVVRFDDD NSPSFIQIRS VAKKHPKTWV HYIAAEEEDW DYAPLVLAPD
 501    DRSYKSQYLN NGPQRIGRKY KKVRFMAYTD ETFKTREAIQ HESGILGPLL
 551    YGEVGDTLLI IFKNQASRPY NIYPHGITDV RPLYSRRLPK GVKHLKDFPI
 601    LPGEIFKYKW TVTVEDGPTK SDPRCLTRYY SSFVNMERDL ASGLIGPLLI

 651    CYKESVDQRG NQIMSDKRNV ILFSVFDENR SWYLTENIQR FLPNPAGVQL
 701    EDPEFQASNI MHSINGYVFD SLQLSVCLHE VAYWYILSIG AQTDFLSVFF
 751    SGYTFKHKMV YEDTLTLFPF SGETVFMSME NPGLWILGCH NSDFRNRGMT
 801    ALLKVSSCDK NTGDYYEDSY EDISAYLLSK NNAIEPRSFS QNGAPGTSES
 851    ATPESGPGSE PATSGSETPG TSESATPESG PGSEPATSGS ETPGTSESAT
 901    PESGPGTSTE PSEGSAPGSP AGSPTSTEEG TSESATPESG PGSEPATSGS
 951    ETPGTSESAT PESGPGSPAG SPTSTEEGSP AGSPTSTEEG TSTEPSEGSA
1001    PGTSESATPE SGPGTSESAT PESGPGTSES ATPESGPGSE PATSGSETPG
1051    SEPATSGSET PGSPAGSPTS TEEGTSTEPS EGSAPGTSTE PSEGSAPGSE
1101    PATSGSETPG TSESATPESG PGTSTEPSEG SAPASSPPVL KRHQAEITRT
1151    TLQSDQEEID YDDTISVEMK KEDFDIYDED ENQSPRSFQK KTRHYFIAAV
1201    ERLWDYGMSS SPHVLRNRAQ SGSVPQFKKV VFQEFTDGSF TQPLYRGELN
1251    EHLGLLGPYI RAEVEDNIMV TFRNQASRPY SFYSSLISYE EDQRQGAEPR
1301    KNFVKPNETK TYFWKVQHHM APTKDEFDCK AWAYFSDVDL EKDVHSGLIG
1351    PLLVCHTNTL NPAHGRQVTV QEFALFFTIF DETKSWYFTE NMERNCRAPC
1401    NIQMEDPTFK ENYRFHAING YIMDTLPGLV MAQDQRIRWY LLSMGSNENI
1451    HSIHFSGHVF TVRKKEEYKM ALYNLYPGVF ETVEMLPSKA GIWRVECLIG
1501    EHLHAGMSTL FLVYSNKCQT PLGMASGHIR DFQITASGQY GQWAPKLARL
1551    HYSGSINAWS TKEPFSWIKV DLLAPMIIHG IKTQGARQKF SSLYISQFII
1601    MYSLDGKKWQ TYRGNSTGTL MVFFGNVDSS GIKHNIFNPP IIARYIRLHP
1651    THYSIRSTLR MELMGCDLNS CSMPLGMESK AISDAQITAS SYFTNMFATW
1701    SPSKARLHLQ GRSNAWRPQV NNPKEWLQVD FQKTMKVTGV TTQGVKSLLT
1751    SMYVKEFLIS SSQDGHQWTL FFQNGKVKVF QGNQDSFTPV VNSLDPPLLT
1801    RYLRIHPQSW VHQIALRMEV LGCEAQDLYD KTHTCPPCPA PELLGGPSVF
1851    LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
1901    REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
1951    QPREPQVYTL PPSRDELTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY
2001    KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
2051    SLSPGK*
```

pSYN FVIII 268 protein sequence (FVIII Fc with 144 AE-XTEN at amino acid 18) SEQ ID NO: 82)

```
   1    MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQGAP TSESATPESG
  51    PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG
 101    TSTEPSEGSA PGSPAGSPTS TEEGTSESAT PESGPGSEPA TSGSETPGTS
 151    ESATPESGPG SPAGSPTSTE EGSPAGSPTS TEEGASSSDL GELPVDARFP
 201    PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 251    DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 301    GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 351    GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 401    HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 451    RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 501    EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 551    WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 601    TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 651    DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 701    YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 751    NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 801    HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 851    MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 901    SKNNAIEPRS FSQNPPVLKR HQAEITRTTL QSDQEEIDYD DTISVEMKKE
 951    DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLRNRAQSG
1001    SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
1051    RNQASRPYSF YSSLISYEED QRQGAEPRKN FVKPNETKTY FWKVQHHMAP
1101    TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
1151    FALFFTIFDE TKSWYFTENM ERNCRAPCNI QMEDPTFKEN YRFHAINGYI
1201    MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
1251    YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL

1301    GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK EPFSWIKVDL
1351    LAPMIIHGIK TQGAROKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
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```
1401    FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
1451    MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
1501    PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISSS QDGHQWTLFF
1551    QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH QIALRMEVLG
1601    CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
1651    VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
1701    LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
1751    SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
1801    KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*
```

pSYN FVIII 269 protein sequence (FVIII Fc with 72 AE-XTEN at amino acid 18) SEQ ID NO: 83)

```
   1    MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQGAP TSESATPESG
  51    PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG
 101    TSTEPSEGSA PGASSSDLGE LPVDARFPPR VPKSFPFNTS VVYKKTLFVE
 151    FTDHLFNIAK PRPPWMGLLG PTIQAEVYDT VVITLKNMAS HPVSLHAVGV
 201    SYWKASEGAE YDDQTSQREK EDDKVFPGGS HTYVWQVLKE NGPMASDPLC
 251    LTYSYLSHVD LVKDLNSGLI GALLVCREGS LAKEKTQTLH KFILLFAVFD
 301    EGKSWHSETK NSLMQDRDAA SARAWPKMHT VNGYVNRSLP GLIGCHRKSV
 351    YWHVIGMGTT PEVHSIFLEG HTFLVRNHRQ ASLEISPITF LTAQTLLMDL
 401    GQFLLFCHIS SHQHDGMEAY VKVDSCPEEP QLRMKNNEEA EDYDDDLTDS
 451    EMDVVRFDDD NSPSFIQIRS VAKKHPKTWV HYIAAEEEDW DYAPLVLAPD
 501    DRSYKSQYLN NGPQRIGRKY KKVRFMAYTD ETFKTREAIQ HESGILGPLL
 551    YGEVGDTLLI IFKNQASRPY NIYPHGITDV RPLYSRRLPK GVKHLKDFPI
 601    LPGEIFKYKW TVTVEDGPTK SDPRCLTRYY SSFVNMERDL ASGLIGPLLI
 651    CYKESVDQRG NQIMSDKRNV ILFSVFDENR SWYLTENIQR FLPNPAGVQL
 701    EDPEFQASNI MHSINGYVFD SLQLSVCLHE VAYWYILSIG AQTDFLSVFF
 751    SGYTFKHKMV YEDTLTLFPF SGETVFMSME NPGLWILGCH NSDFRNRGMT
 801    ALLKVSSCDK NTGDYYEDSY EDISAYLLSK NNAIEPRSFS QNPPVLKRHQ
 851    AEITRTTLQS DQEEIDYDDT ISVEMKKEDF DIYDEDENQS PRSFQKKTRH
 901    YFIAAVERLW DYGMSSSPHV LRNRAQSGSV PQFKKVVFQE FTDGSFTQPL
 951    YRGELNEHLG LLGPYIRAEV EDNIMVTFRN QASRPYSFYS SLISYEEDQR
1001    QGAEPRKNFV KPNETKTYFW KVQHHMAPTK DEFDCKAWAY FSDVDLEKDV
1051    HSGLIGPLLV CHTNTLNPAH GRQVTVQEFA LFFTIFDETK SWYFTENMER
1101    NCRAPCNIQM EDPTFKENYR FHAINGYIMD TLPGLVMAQD QRIRWYLLSM
1151    GSNENIHSIH FSGHVFTVRK KEEYKMALYN LYPGVFETVE MLPSKAGIWR
1201    VECLIGEHLH AGMSTLFLVY SNKCQTPLGM ASGHIRDFQI TASGQYGQWA
1251    PKLARLHYSG SINAWSTKEP FSWIKVDLLA PMIIHGIKTQ GARQKFSSLY
1301    ISQFIIMYSL DGKKWQTYRG NSTGTLMVFF GNVDSSGIKH NIFNPPIIAR
1351    YIRLHPTHYS IRSTLRMELM GCDLNSCSMP LGMESKAISD AQITASSYFT
1401    NMFATWSPSK ARLHLQGRSN AWRPQVNNPK EWLQVDFQKT MKVTGVTTQG
1451    VKSLLTSMYV KEFLISSSQD GHQWTLFFQN GKVKVFQGNQ DSFTPVVNSL
1501    DPPLLTRYLR IHPQSWVHQI ALRMEVLGCE AQDLYDKTHT CPPCPAPELL
1551    GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH
1601    NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT
1651    ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG
1701    QPENNYKTTP PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH
1751    YTQKSLSLSP GK*
```

pSYNFVIII 271 protein sequence (FVIII Fc with 42 AE-XTEN at amino acid 18) SEQ ID NO: 84)

```
1    MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQGAP GSPAGSPTST

  51    EEGTSESATP ESGPGSEPAT SGSETPASSS DLGELPVDAR FPPRVPKSFP
 101    FNTSVVYKKT LFVEFTDHLF NIAKPRPPWM GLLGPTIQAE VYDTVVITLK
 151    NMASHPVSLH AVGVSYWKAS EGAEYDDQTS QREKEDDKVF PGGSHTYVWQ
 201    VLKENGPMAS DPLCLTYSYL SHVDLVKDLN SGLIGALLVC REGSLAKEKT
 251    QTLHKFILLF AVFDEGKSWH SETKNSLMQD RDAASARAWP KMHTVNGYVN
 301    RSLPGLIGCH RKSVYWHVIG MGTTPEVHSI FLEGHTFLVR NHRQASLEIS
 351    PITFLTAQTL LMDLGQFLLF CHISSHQHDG MEAYVKVDSC PEEPQLRMKN
 401    NEEAEDYDDD LTDSEMDVVR FDDDNSPSFI QIRSVAKKHP KTWVHYIAAE
 451    EEDWDYAPLV LAPDDRSYKS QYLNNGPQRI GRKYKKVRFM AYTDETFKTR
 501    EAIQHESGIL GPLLYGEVGD TLLIIFKNQA SRPYNIYPHG ITDVRPLYSR
 551    RLPKGVKHLK DFPILPGEIF KYKWTVTVED GPTKSDPRCL TRYYSSFVNM
 601    ERDLASGLIG PLLICYKESV DQRGNQIMSD KRNVILFSVF DENRSWYLTE
 651    NIQRFLPNPA GVQLEDPEFQ ASNIMHSING YVFDSLQLSV CLHEVAYWYI
 701    LSIGAQTDFL SVFFSGYTFK HKMVYEDTLT LFPFSGETVF MSMENPGLWI
 751    LGCHNSDFRN RGMTALLKVS SCDKNTGDYY EDSYEDISAY LLSKNNAIEP
 801    RSFSQNPPVL KRHQAEITRT TLQSDQEEID YDDTISVEMK KEDFDIYDED
 851    ENQSPRSFQK KTRHYFIAAV ERLWDYGMSS SPHVLRNRAQ SGSVPQFKKV
 901    VFQEFTDGSF TQPLYRGELN EHLGLLGPYI RAEVEDNIMV TFRNQASRPY
 951    SFYSSLISYE EDQRQGAEPR KNFVKPNETK TYFWKVQHHM APTKDEFDCK
1001    AWAYFSDVDL EKDVHSGLIG PLLVCHTNTL NPAHGRQVTV QEFALFFTIF
1051    DETKSWYFTE NMERNCRAPC NIQMEDPTFK ENYRFHAING YIMDTLPGLV
1101    MAQDQRIRWY LLSMGSNENI HSIHFSGHVF TVRKKEEYKM ALYNLYPGVF
1151    ETVEMLPSKA GIWRVECLIG EHLHAGMSTL FLVYSNKCQT PLGMASGHIR
1201    DFQITASGQY GQWAPKLARL HYSGSINAWS TKEPFSWIKV DLLAPMIIHG
1251    IKTQGARQKF SSLYISQFII MYSLDGKKWQ TYRGNSTGTL MVFFGNVDSS
1301    GIKHNIFNPP IIARYIRLHP THYSIRSTLR MELMGCDLNS CSMPLGMESK
1351    AISDAQITAS SYFTNMFATW SPSKARLHLQ GRSNAWRPQV NNPKEWLQVD
1401    FQKTMKVTGV TTQGVKSLLT SMYVKEFLIS SSQDGHQWTL FFQNGKVKVF
1451    QGNQDSFTPV VNSLDPPLLT RYLRIHPQSW VHQIALRMEV LGCEAQDLYD
1501    KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
1551    EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC
1601    KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN QVSLTCLVKG
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1601    KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN QVSLTCLVKG
1651    FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN
1701    VFSCSVMHEA LHNHYTQKSL SLSPGK*
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pSYN FVIII protein sequence 272 (FVIII with 144 AE XTEN at amino acid 18 and 244 AE XTEN in B-domain- no Fc) SEQ ID NO: 85)

```
   1    MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQGAP TSESATPESG
  51    PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG
 101    TSTEPSEGSA PGSPAGSPTS TEEGTSESAT PESGPGSEPA TSGSETPGTS
 151    ESATPESGPG SPAGSPTSTE EGSPAGSPTS TEEGASSSDL GELPVDARFP
 201    PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 251    DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 301    GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 351    GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 401    HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 451    RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 501    EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 551    WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 601    TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 651    DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 701    YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 751    NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 801    HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 851    MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 901    SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSESATPE
 951    SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE

1001    EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP AGSPTSTEEG
1051    SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
1101    ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
1151    PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPGTSTEPS
1201    EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
1251    EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVPQFK
1301    KVVFQEFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
1351    PYSFYSSLIS YEEDQRQGAE PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
1401    CKAWAYFSDV DLEKDVHSGL IGPLLVCHTN TLNPAHGRQV TVQEFALFFT
1451    IFDETKSWYF TENMERNCRA PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
1501    LVMAQDQRIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
1551    VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
1601    IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
1651    HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
1701    SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
1751    SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
1801    VDFQKTMKVT GVTTQGVKSL LTSMYVKEFL ISSSQDGHQW TLFFQNGKVK
1851    VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
1901    Y*
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pSYN-FVIII-161 protein sequence (SEQ ID NO: 69) (FVIII sequence amino acid position 1-1457; underlined region represents Fc region; curvy underline represents cleavable linker in between first Fc and VWF fragment; double underlined region represents VWF fragment; bold region represents cleavable linker in between VWF fragment and Fc).

```
   1    MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP
  51    PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101    DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 151    GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201    GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251    HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301    RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351    EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401    WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451    TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 501    DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551    YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601    NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651    HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 701    MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 751    SKNNAIEPRS FSQNPPVLKR HQREITRTTL QSDQEEIDYD DTISVEMKKE
 801    DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLRNRAQSG
 851    SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
 901    RNQASRPYSF YSSLISYEED QRQGAEPRKN FVKPNETKTY FWKVQHHMAP
 951    TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
1001    FALFFTIFDE TKSWYFTENM ERNCRAPCNI QMEDPTFKEN YRFHAINGYI
1051    MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
1101    YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
1151    GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK EPFSWIKVDL
1201    LAPMIIHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
1251    FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
1301    MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
1351    PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISSS QDGHQWTLFF
1401    QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH QIALRMEVLG
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1451    CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
1501    VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
1551    LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
1601    SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
1651    KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGKRRRRSG GGGSGGGGSG
1701    GGGSGGGGSG GGGSGGGGSR KRRKRSLSCR PPMVKLVCPA DNLRAEGLEC
1751    TKTCQNYDLE CMSMGCVSGC LCPPGMVRHE NRCVALERCP CFHQGKEYAP
1801    GETVKIGCNT CVCRDRKWNC TDHVCDATCS TIGMAHYLTF DGLKYLFPGE
1851    CQYVLVQDYC GSNPGTFRIL VGNKGCSHPS VKCKKRVTIL VEGGEIELFD
1901    GEVNVKRPMK DETHFEVVES GRYIILLLGK ALSVVWDRHL SISVVLKQTY
1951    QEKVCGLCGN FDGIQNNDLT SSNLQVEEDP VDFGNSWKVS SQCADTRKVP
2001    LDSSPATCHN NIMKQTMVDS SCRILTSDVF QDCNKLVDPE PYLDVCIYDT
2051    CSCESIGDCA AFCDTIAAYA HVCAQHGKVV TWRTATLCPQ SCEERNLREN
2101    GYEAEWRYNS CAPACQVTCQ HPEPLACPVQ CVEGCHAHCP PGKILDELLQ
2151    TCVDPEDCPV CEVAGRRFAS GKKVTLNPSD PEHCQICHCD VVNLTCEACQ
2201    EPISGTSESA TPESGPGSEP ATSGSETPGT SESATPESGP GSEPATSGSE
2251    TPGTSESATP ESGPGTSTEP SEGSAPGSPA GSPTSTEEGT SESATPESGP
2301    GSEPATSGSE TPGTSESATP ESGPGSPAGS PTSTEEGSPA GSPTSTEEGT
2351    STEPSEGSAP GTSESATPES GPGTSESATP ESGPGTSESA TPESGPGSEP
2401    ATSGSETPGS EPATSGSETP GSPAGSPTST EEGTSTEPSE GSAPGTSTEP
2451    SEGSAPGSEP ATSGSETPGT SESATPESGP GTSTEPSEGS APDSGGGGSG
2501    GGGSGGGGSG GGGSGGGGSL VPRGSGGDKT HTCPPCPAPE LLGGPSVFLF
2551    PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE
2601    EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
2651    REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT
2701    TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
2751        SPGK
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pSYN-FVIII-170 protein sequence (SEQ ID NO: 71)

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   1  SLSCRPPMVK LVCPADNLRA EGLECTKTCQ NYDLECMSMG CVSGCLCPPG
  51  MVRHENRCVA LERCPCFHQG KEYAPGETVK IGCNTCVCRD RKWNCTDHVC
 101  DATCSTIGMA HYLTFDGLKY LFPGECQYVL VQDYCGSNPG TFRILVGNKG
 151  CSHPSVKCKK RVTILVEGGE IELFDGEVNV KRPMKDETHF EVVESGRYII
 201  LLLGKALSVV WDRHLSISVV LKQTYQEKVC GLCGNFDGIQ NNDLTSSNLQ
 251  VEEDPVDFGN SWKVSSQCAD TRKVPLDSSP ATCHNNIMKQ TMVDSSCRIL
 301  TSDVFQDCNK LVDPEPYLDV CIYDTCSCES IGDCAAFCDT IAAYAHVCAQ
 351  HGKVVTWRTA TLCPQSCEER NLRENGYEAE WRYNSCAPAC QVTCQHPEPL
 401  ACPVQCVEGC HAHCPPGKIL DELLQTCVDP EDCPVCEVAG RRFASGKKVT
 451  LNPSDPEHCQ ICHCDVVNLT CEACQEPISG TSESATPESG PGSEPATSGS
 501  ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG TSTEPSEGSA
 551  PGSPAGSPTS TEEGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG
 601  SPAGSPTSTE EGSPAGSPTS TEEGTSTEPS EGSAPGTSES ATPESGPGTS
 651  ESATPESGPG TSESATPESG PGSEPATSGS ETPGSEPATS GSETPGSPAG
 701  SPTSTEEGTS TEPSEGSAPG TSTEPSEGSA PGSEPATSGS ETPGTSESAT
 751  PESGPGTSTE PSEGSAPDSG GGGSGGGGSG GGGSGGGGSG GGGSLVPRGS
 801  GGASATRRYY LGAVELSWDY MQSDLGELPV DARFPPRVPK SFPFNTSVVY
 851  KKTLFVEFTD HLFNIAKPRP PWMGLLGPTI QAEVYDTVVI TLKNMASHPV
 901  SLHAVGVSYW KASEGAEYDD QTSQREKEDD KVFPGGSHTY VWQVLKENGP
 951  MASDPLCLTY SYLSHVDLVK DLNSGLIGAL LVCREGSLAK EKTQTLHKFI
1001  LLFAVFDEGK SWHSETKNSL MQDRDAASAR AWPKMHTVNG YVNRSLPGLI
1051  GCHRKSVYWH VIGMGTTPEV HSIFLEGHTF LVRNHRQASL EISPITFLTA
1101  QTLLMDLGQF LLFCHISSHQ HDGMEAYVKV DSCPEEPQLR MKNNEEAEDY
1151  DDDLTDSEMD VVRFDDDNSP SFIQIRSVAK KHPKTWVHYI AAEEEDWDYA
1201  PLVLAPDDRS YKSQYLNNGP QRIGRKYKKV RFMAYTDETF KTREAIQHES
1251  GILGPLLYGE VGDTLLIIFK NQASRPYNIY PHGITDVRPL YSRRLPKGVK
1301  HLKDFPILPG EIFKYKWTVT VEDGPTKSDP RCLTRYYSSF VNMERDLASG
1351  LIGPLLICYK ESVDQRGNQI MSDKRNVILF SVFDENRSWY LTENIQRFLP
1401  NPAGVQLEDP EFQASNIMHS INGYVFDSLQ LSVCLHEVAY WYILSIGAQT

1451  DFLSVFFSGY TFKHKMVYED TLTLFPFSGE TVFMSMENPG LWILGCHNSD
1501  FRNRGMTALL KVSSCDKNTG DYYEDSYEDI SAYLLSKNNA IEPRSFSQNP
1551  PVLKRHQREI TRTTLQSDQE EIDYDDTISV EMKKEDFDIY DEDENQSPRS
1601  FQKKTRHYFI AAVERLWDYG MSSSPHVLRN RAQSGSVPQF KKVVFQEFTD
1651  GSFTQPLYRG ELNEHLGLLG PYIRAEVEDN IMVTFRNQAS RPYSFYSSLI
1701  SYEEDQRQGA EPRKNFVKPN ETKTYFWKVQ HHMAPTKDEF DCKAWAYFSD
1751  VDLEKDVHSG LIGPLLVCHT NTLNPAHGRQ VTVQEFALFF TIFDETKSWY
1801  FTENMERNCR APCNIQMEDP TFKENYRFHA INGYIMDTLP GLVMAQDQRI
1851  RWYLLSMGSN ENIHSIHFSG HVFTVRKKEE YKMALYNLYP GVFETVEMLP
1901  SKAGIWRVEC LIGEHLHAGM STLFLVYSNK CQTPLGMASG HIRDFQITAS
1951  GQYGQWAPKL ARLHYSGSIN AWSTKEPFSW IKVDLLAPMI IHGIKTQGAR
2001  QKFSSLYISQ FIIMYSLDGK KWQTYRGNST GTLMVFFGNV DSSGIKHNIF
2051  NPPIIARYIR LHPTHYSIRS TLRMELMGCD LNSCSMPLGM ESKAISDAQI
2101  TASSYFTNMF ATWSPSKARL HLQGRSNAWR PQVNNPKEWL QVDFQKTMKV
2151  TGVTTQGVKS LLTSMYVKEF LISSSQDGHQ WTLFFQNGKV KVFQGNQDSF
2201  TPVVNSLDPP LLTRYLRIHP QSWVHQIALR MEVLGCEAQD LY
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pSYN FVIII 310 nucleotide sequence (encoding FVIII with complete B-domain deletion except 2 amino acid residues and 288 AE-XTEN inserted after aa 742) (SEQ ID NO:170)

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   1    ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT TGCGATTCTG
  51    CTTTAGTGCC ACCAGAAGAT ACTACCTGGG TGCAGTGGAA CTGTCATGGG
 101    ACTATATGCA AAGTGATCTC GGTGAGCTGC CTGTGGACGC AAGATTTCCT
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 151    CCTAGAGTGC CAAAATCTTT TCCATTCAAC ACCTCAGTCG TGTACAAAAA
 201    GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC GCTAAGCCAA
 251    GGCCACCCTG GATGGGTCTG CTAGGTCCTA CCATCCAGGC TGAGGTTTAT
 301    GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTCAGTCT
 351    TCATGCTGTT GGTGTATCCT ACTGGAAAGC TTCTGAGGGA GCTGAATATG
 401    ATGATCAGAC CAGTCAAAGG GAGAAAGAAG ATGATAAAGT CTTCCCTGGT
 451    GGAAGCCATA CATATGTCTG GCAGGTCCTG AAAGAGAATG GTCCAATGGC
 501    CTCTGACCCA CTGTGCCTTA CCTACTCATA TCTTTCTCAT GTGGACCTGG
 551    TAAAAGACTT GAATTCAGGC CTCATTGGAG CCCTACTAGT ATGTAGAGAA
 601    GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT
 651    TTTTGCTGTA TTTGATGAAG GGAAAAGTTG GCACTCAGAA ACAAAGAACT
 701    CCTTGATGCA GGATAGGGAT GCTGCATCTG CTCGGGCCTG GCCTAAAATG
 751    CACACAGTCA ATGGTTATGT AAACAGGTCT CTGCCAGGTC TGATTGGATG
 801    CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAATGGGC ACCACTCCTG
 851    AAGTGCACTC AATATTCCTC GAAGGTCACA CATTTCTTGT GAGGAACCAT
 901    CGCCAGGCGT CCTTGGAAAT CTCGCCAATA ACTTTCCTTA CTGCTCAAAC
 951    ACTCTTGATG GACCTTGGAC AGTTTCTACT GTTTTGTCAT ATCTCTTCCC
1001    ACCAACATGA TGGCATGGAA GCTTATGTCA AAGTAGACAG CTGTCCAGAG
1051    GAACCCCAAC TACGAATGAA AAATAATGAA GAAGCGGAAG ACTATGATGA
1101    TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT GATGACAACT
1151    CTCCTTCCTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA TCCTAAAACT
1201    TGGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCTT
1251    AGTCCTCGCC CCCGATGACA GAAGTTATAA AAGTCAATAT TTGAACAATG
1301    GCCCTCAGCG GATTGGTAGG AAGTACAAAA AAGTCCGATT TATGGCATAC
1351    ACAGATGAAA CCTTTAAGAC TCGTGAAGCT ATTCAGCATG AATCAGGAAT
1401    CTTGGGACCT TTACTTTATG GGGAAGTTGG AGACACACTG TTGATTATAT
1451    TTAAGAATCA AGCAAGCAGA CCATATAACA TCTACCCTCA CGGAATCACT
1501    GATGTCCGTC CTTTGTATTC AAGGAGATTA CCAAAAGGTG TAAAACATTT
1551    GAAGGATTTT CCAATTCTGC CAGGAGAAAT ATTCAAATAT AAATGGACAG
1601    TGACTGTAGA AGATGGGCCA ACTAAATCAG ATCCTCGGTG CCTGACCCGC
1651    TATTACTCTA GTTTCGTTAA TATGGAGAGA GATCTAGCTT CAGGACTCAT
1701    TGGCCCTCTC CTCATCTGCT ACAAAGAATC TGTAGATCAA AGAGGAAACC
1751    AGATAATGTC AGACAAGAGG AATGTCATCC TGTTTTCTGT ATTTGATGAG
1801    AACCGAAGCT GGTACCTCAC AGAGAATATA CAACGCTTTC TCCCCAATCC

1851    AGCTGGAGTG CAGCTTGAGG ATCCAGAGTT CCAAGCCTCC AACATCATGC
1901    ACAGCATCAA TGGCTATGTT TTTGATAGTT TGCAGTTGTC AGTTTGTTTG
1951    CATGAGGTGG CATACTGGTA CATTCTAAGC ATTGGAGCAC AGACTGACTT
2001    CCTTTCTGTC TTCTTCTCTG GATATACCTT CAAACACAAA ATGGTCTATG
2051    AAGACACACT CACCCTATTC CCATTCTCAG GAGAAACTGT CTTCATGTCG
2101    ATGGAAAACC CAGGTCTATG GATTCTGGGG TGCCACAACT CAGACTTTCG
2151    GAACAGAGGC ATGACCGCCT TACTGAAGGT TTCTAGTTGT GACAAGAACA
2201    CTGGTGATTA TTACGAGGAC AGTTATGAAG ATATTTCAGC ATACTTGCTG
2251    AGTAAAAACA ATGCCATTGA ACCAAGAAGC TTCGGTACCT CAGAGTCTGC
2301    TACCCCCGAG TCAGGGCCAG GATCAGAGCC AGCCACCTCC GGGTCTGAGA
2351    CACCCGGGAC TTCCGAGAGT GCCACCCCTG AGTCCGGACC CGGGTCCGAG
2401    CCCGCCACTT CCGGCTCCGA AACTCCCGGC ACAAGCGAGA GCGCTACCCC
2451    AGAGTCAGGA CCAGGAACAT CTACAGAGCC CTCTGAAGGC TCCGCTCCAG
2501    GGTCCCCAGC CGGCAGTCCC ACTAGCACCG AGGAGGGAAC CTCTGAAAGC
2551    GCCACACCCG AATCAGGGCC AGGGTCTGAG CCTGCTACCA GCGGCAGCGA
2601    GACACCAGGC ACCTCTGAGT CCGCCACACC AGAGTCCGGA CCCGGATCTC
2651    CCGCTGGGAG CCCCACCTCC ACTGAGGAGG GATCTCCTGC TGGCTCTCCA
2701    ACATCTACTG AGGAAGGTAC CTCAACCGAG CCATCCGAGG GATCAGCTCC
2751    CGGCACCTCA GAGTCGGCAA CCCCGGAGTC TGGACCCGGA ACTTCCGAAA
2801    GTGCCACACC AGAGTCCGGT CCCGGGACTT CAGAATCAGC AACACCCGAG
2851    TCCGGCCCTG GGTCTGAACC CGCCACAAGT GGTAGTGAGA CACCAGGATC
2901    AGAACCTGCT ACCTCAGGGT CAGAGACACC CGGATCTCCG GCAGGCTCAC
2951    CAACCTCCAC TGAGGAGGGC ACCAGCACAG AACCAAGCGA GGGCTCCGCA
3001    CCCGGAACAA GCACTGAACC CAGTGAGGGT TCAGCACCCG GCTCTGAGCC
3051    GGCCACAAGT GGCAGTGAGA CACCCGGCAC TTCAGAGAGT GCCACCCCCG
3101    AGAGTGGCCC AGGCACTAGT ACCGAGCCCT CTGAAGGCAG TGCGCCAGCC
3151    TCGAGCGAAA TAACTCGTAC TACTCTTCAG TCAGATCAAG AGGAAATCGA
3201    TTATGATGAT ACCATATCAG TTGAAATGAA GAAGGAAGAT TTTGACATTT
3251    ATGATGAGGA TGAAAATCAG AGCCCCCGCA GCTTTCAAAA GAAAACACGA
3301    CACTATTTTA TTGCTGCAGT GGAGAGGCTC TGGGATTATG GGATGAGTAG
3351    CTCCCCACAT GTTCTAAGAA ACAGGGCTCA GAGTGGCAGT GTCCCTCAGT
3401    TCAAGAAAGT TGTTTTCCAG GAATTTACTG ATGGCTCCTT TACTCAGCCC
3451    TTATACCGTG GAGAACTAAA TGAACATTTG GGACTCCTGG GGCCATATAT
3501    AAGAGCAGAA GTTGAAGATA ATATCATGGT AACTTTCAGA AATCAGGCCT
3551    CTCGTCCCTA TTCCTTCTAT TCTAGCCTTA TTTCTTATGA GGAAGATCAG
3601    AGGCAAGGAG CAGAACCTAG AAAAAACTTT GTCAAGCCTA ATGAAACCAA
3651    AACTTACTTT TGGAAAGTGC AACATCATAT GGCACCCACT AAAGATGAGT
3701    TTGACTGCAA AGCCTGGGCT TATTTCTCTG ATGTTGACCT GGAAAAAGAT
3751    GTGCACTCAG GCCTGATTGG ACCCCTTCTG GTCTGCCACA CTAACACACT
3801    GAACCCTGCT CATGGGAGAC AAGTGACAGT ACAGGAATTT GCTCTGTTTT
3851    TCACCATCTT TGATGAGACC AAAAGCTGGT ACTTCACTGA AAATATGGAA
3901    AGAAACTGCA GGGCTCCCTG CAATATCCAG ATGGAAGATC CCACTTTTAA
3951    AGAGAATTAT CGCTTCCATG CAATCAATGG CTACATAATG GATACACTAC
4001    CTGGCTTAGT AATGGCTCAG GATCAAAGGA TTCGATGGTA TCTGCTCAGC
4051    ATGGGCAGCA ATGAAAACAT CCATTCTATT CATTTCAGTG GACATGTGTT
4101    CACTGTACGA AAAAAAGAGG AGTATAAAAT GGCACTGTAC AATCTCTATC
4151    CAGGTGTTTT TGAGACAGTG GAAATGTTAC CATCCAAAGC TGGAATTTGG
4201    CGGGTGGAAT GCCTTATTGG CGAGCATCTA CATGCTGGGA TGAGCACACT
4251    TTTTCTGGTG TACAGCAATA AGTGTCAGAC TCCCCTGGGA ATGGCTTCTG
4301    GACACATTAG AGATTTTCAG ATTACAGCTT CAGGACAATA TGGACAGTGG
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4351    GCCCCAAAGC TGGCCAGACT TCATTATTCC GGATCAATCA ATGCCTGGAG
4401    CACCAAGGAG CCCTTTTCTT GGATCAAGGT GGATCTGTTG GCACCAATGA
4451    TTATTCACGG CATCAAGACC CAGGGTGCCC GTCAGAAGTT CTCCAGCCTC
4501    TACATCTCTC AGTTTATCAT CATGTATAGT CTTGATGGGA AGAAGTGGCA
4551    GACTTATCGA GGAAATTCCA CTGGAACCTT AATGGTCTTC TTTGGCAATG
4601    TGGATTCATC TGGGATAAAA CACAATATTT TTAACCCTCC AATTATTGCT
4651    CGATACATCC GTTTGCACCC AACTCATTAT AGCATTCGCA GCACTCTTCG
4701    CATGGAGTTG ATGGGCTGTG ATTTAAATAG TTGCAGCATG CCATTGGGAA
4751    TGGAGAGTAA AGCAATATCA GATGCACAGA TTACTGCTTC ATCCTACTTT

4801    ACCAATATGT TTGCCACCTG GTCTCCTTCA AAAGCTCGAC TTCACCTCCA
4851    AGGGAGGAGT AATGCCTGGA GACCTCAGGT GAATAATCCA AAAGAGTGGC
4901    TGCAAGTGGA CTTCCAGAAG ACAATGAAAG TCACAGGAGT AACTACTCAG
4951    GGAGTAAAAT CTCTGCTTAC CAGCATGTAT GTGAAGGAGT TCCTCATCTC
5001    CAGCAGTCAA GATGGCCATC AGTGGACTCT CTTTTTTCAG AATGGCAAAG
5051    TAAAGGTTTT TCAGGGAAAT CAAGACTCCT TCACACCTGT GGTGAACTCT
5101    CTAGACCCAC CGTTACTGAC TCGCTACCTT CGAATTCACC CCCAGAGTTG
5151    GGTGCACCAG ATTGCCCTGA GGATGGAGGT TCTGGGCTGC GAGGCACAGG
5201    ACCTCTACGA CAAAACTCAC ACATGCCCAC CGTGCCCAGC TCCAGAACTC
5251    CTGGGCGGAC CGTCAGTCTT CCTCTTCCCC CCAAAACCCA AGGACACCCT
5301    CATGATCTCC CGGACCCCTG AGGTCACATG CGTGGTGGTG GACGTGAGCC
5351    ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG
5401    CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG
5451    TGTGGTCAGC GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG
5501    AGTACAAGTG CAAGGTCTCC AACAAAGCCC TCCCAGCCCC CATCGAGAAA
5551    ACCATCTCCA AAGCCAAAGG GCAGCCCCGA GAACCACAGG TGTACACCCT
5601    GCCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC CTGACCTGCC
5651    TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT
5701    GGGCAGCCGG AGAACAACTA CAAGACCACG CCTCCCGTGT TGGACTCCGA
5751    CGGCTCCTTC TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC
5801    AGCAGGGGAA CGTCTTCTCA TGCTCCGTGA TGCATGAGGC TCTGCACAAC
5851    CACTACACGC AGAAGAGCCT CTCCCTGTCT CCGGGTAAAT GA
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pSYN FVIII 310 protein sequence (FVIII with complete B-domain deletion except 2 amino acid residues and 288 AE-XTEN inserted after aa 742) (SEQ ID NO:171)

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   1    ATRRYYLGAV ELSWDYMQSD LGELPVDARF PPRVPKSFPF NTSVVYKKTL
  51    FVEFTDHLFN IAKPRPPWMG LLGPTIQAEV YDTVVITLKN MASHPVSLHA
 101    VGVSYWKASE GAEYDDQTSQ REKEDDKVFP GGSHTYVWQV LKENGPMASD
 151    PLCLTYSYLS HVDLVKDLNS GLIGALLVCR EGSLAKEKTQ TLHKFILLFA
 201    VFDEGKSWHS ETKNSLMQDR DAASARAWPK MHTVNGYVNR SLPGLIGCHR
 251    KSVYWHVIGM GTTPEVHSIF LEGHTFLVRN HRQASLEISP ITFLTAQTLL
 301    MDLGQFLLFC HISSHQHDGM EAYVKVDSCP EEPQLRMKNN EEAEDYDDDL
 351    TDSEMDVVRF DDDNSPSFIQ IRSVAKKHPK TWVHYIAAEE EDWDYAPLVL
 401    APDDRSYKSQ YLNNGPQRIG RKYKKVRFMA YTDETFKTRE AIQHESGILG
 451    PLLYGEVGDT LLIIFKNQAS RPYNIYPHGI TDVRPLYSRR LPKGVKHLKD
 501    FPILPGEIFK YKWTVTVEDG PTKSDPRCLT RYYSSFVNME RDLASGLIGP
 551    LLICYKESVD QRGNQIMSDK RNVILFSVFD ENRSWYLTEN IQRFLPNPAG
 601    VQLEDPEFQA SNIMHSINGY VFDSLQLSVC LHEVAYWYIL SIGAQTDFLS
 651    VFFSGYTFKH KMVYEDTLTL FPFSGETVFM SMENPGLWIL GCHNSDFRNR
 701    GMTALLKVSS CDKNTGDYYE DSYEDISAYL LSKNNAIEPR SFGTSESATP
 751    ESGPGSEPAT SGSETPGTSE SATPESGPGS EPATSGSETP GTSESATPES
 801    GPGTSTEPSE GSAPGSPAGS PTSTEEGTSE SATPESGPGS EPATSGSETP
 851    GTSESATPES GPGSPAGSPT STEEGSPAGS PTSTEEGTST EPSEGSAPGT
 901    SESATPESGP GTSESATPES GPGTSESATP ESGPGSEPAT SGSETPGSEP
 951    ATSGSETPGS PAGSPTSTEE GTSTEPSEGS APGTSTEPSE GSAPGSEPAT
1001    SGSETPGTSE SATPESGPGT STEPSEGSAP ASSEITRTTL QSDQEEIDYD
1051    DTISVEMKKE DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP
1101    HVLRNRAQSG SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA
1151    EVEDNIMVTF RNQASRPYSF YSSLISYEED QRQGAEPRKN FVKPNETKTY
1201    FWKVQHHMAP TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP
1251    AHGRQVTVQE FALFFTIFDE TKSWYFTENM ERNCRAPCNI QMEDPTFKEN
1301    YRFHAINGYI MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV
1351    RKKEEYKMAL YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL
1401    VYSNKCQTPL GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK
1451    EPFSWIKVDL LAPMIIHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY
1501    RGNSTGTLMV FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME

1551    LMGCDLNSCS MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR
1601    SNAWRPQVNN PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISSS
1651    QDGHQWTLFF QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH
1701    QIALRMEVLG CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI
1751    SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV
1801    SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP
1851    SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS
1901    FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*
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pSYN FVIII 312 nucleotide sequence (encoding FVIII with complete B-domain deletion except 5 amino acid residues and 288 AE-XTEN inserted after aa 745- B5 version) (SEQ ID NO:172

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   1    ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT TGCGATTCTG
  51    CTTTAGTGCC ACCAGAAGAT ACTACCTGGG TGCAGTGGAA CTGTCATGGG
 101    ACTATATGCA AAGTGATCTC GGTGAGCTGC CTGTGGACGC AAGATTTCCT
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 151    CCTAGAGTGC CAAAATCTTT TCCATTCAAC ACCTCAGTCG TGTACAAAAA
 201    GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC GCTAAGCCAA
 251    GGCCACCCTG GATGGGTCTG CTAGGTCCTA CCATCCAGGC TGAGGTTTAT
 301    GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTCAGTCT
 351    TCATGCTGTT GGTGTATCCT ACTGGAAAGC TTCTGAGGGA GCTGAATATG
 401    ATGATCAGAC CAGTCAAAGG GAGAAAGAAG ATGATAAAGT CTTCCCTGGT
 451    GGAAGCCATA CATATGTCTG GCAGGTCCTG AAAGAGAATG GTCCAATGGC
 501    CTCTGACCCA CTGTGCCTTA CCTACTCATA TCTTTCTCAT GTGGACCTGG
 551    TAAAAGACTT GAATTCAGGC CTCATTGGAG CCCTACTAGT ATGTAGAGAA
 601    GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT
 651    TTTTGCTGTA TTTGATGAAG GGAAAAGTTG GCACTCAGAA ACAAAGAACT
 701    CCTTGATGCA GGATAGGGAT GCTGCATCTG CTCGGGCCTG GCCTAAAATG
 751    CACACAGTCA ATGGTTATGT AAACAGGTCT CTGCCAGGTC TGATTGGATG
 801    CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAATGGGC ACCACTCCTG
 851    AAGTGCACTC AATATTCCTC GAAGGTCACA CATTTCTTGT GAGGAACCAT
 901    CGCCAGGCGT CCTTGGAAAT CTCGCCAATA ACTTTCCTTA CTGCTCAAAC
 951    ACTCTTGATG GACCTTGGAC AGTTTCTACT GTTTTGTCAT ATCTCTTCCC
1001    ACCAACATGA TGGCATGGAA GCTTATGTCA AAGTAGACAG CTGTCCAGAG
1051    GAACCCCAAC TACGAATGAA AAATAATGAA GAAGCGGAAG ACTATGATGA
1101    TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT GATGACAACT
1151    CTCCTTCCTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA TCCTAAAACT
1201    TGGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCTT
1251    AGTCCTCGCC CCCGATGACA GAAGTTATAA AAGTCAATAT TTGAACAATG
1301    GCCCTCAGCG GATTGGTAGG AAGTACAAAA AAGTCCGATT TATGGCATAC
1351    ACAGATGAAA CCTTTAAGAC TCGTGAAGCT ATTCAGCATG AATCAGGAAT
1401    CTTGGGACCT TTACTTTATG GGGAAGTTGG AGACACACTG TTGATTATAT
1451    TTAAGAATCA AGCAAGCAGA CCATATAACA TCTACCCTCA CGGAATCACT
1501    GATGTCCGTC CTTTGTATTC AAGGAGATTA CCAAAAGGTG TAAAACATTT
1551    GAAGGATTTT CCAATTCTGC CAGGAGAAAT ATTCAAATAT AAATGGACAG
1601    TGACTGTAGA AGATGGGCCA ACTAAATCAG ATCCTCGGTG CCTGACCCGC
1651    TATTACTCTA GTTTCGTTAA TATGGAGAGA GATCTAGCTT CAGGACTCAT
1701    TGGCCCTCTC CTCATCTGCT ACAAAGAATC TGTAGATCAA AGAGGAAACC
1751    AGATAATGTC AGACAAGAGG AATGTCATCC TGTTTTCTGT ATTTGATGAG
1801    AACCGAAGCT GGTACCTCAC AGAGAATATA CAACGCTTTC TCCCCAATCC
1851    AGCTGGAGTG CAGCTTGAGG ATCCAGAGTT CCAAGCCTCC AACATCATGC
1901    ACAGCATCAA TGGCTATGTT TTTGATAGTT TGCAGTTGTC AGTTTGTTTG
1951    CATGAGGTGG CATACTGGTA CATTCTAAGC ATTGGAGCAC AGACTGACTT
2001    CCTTTCTGTC TTCTTCTCTG GATATACCTT CAAACACAAA ATGGTCTATG
2051    AAGACACACT CACCCTATTC CCATTCTCAG GAGAAACTGT CTTCATGTCG
2101    ATGGAAAACC CAGGTCTATG GATTCTGGGG TGCCACAACT CAGACTTTCG

2151    GAACAGAGGC ATGACCGCCT TACTGAAGGT TTCTAGTTGT GACAAGAACA
2201    CTGGTGATTA TTACGAGGAC AGTTATGAAG ATATTTCAGC ATACTTGCTG
2251    AGTAAAAACA ATGCCATTGA ACCAAGAAGC TTCTCTCAAA ACGGTACCTC
2301    AGAGTCTGCT ACCCCCGAGT CAGGGCCAGG ATCAGAGCCA GCCACCTCCG
2351    GGTCTGAGAC ACCCGGCACT TCCGAGAGTG CCACCCCTGA GTCCGGACCC
2401    GGGTCCGAGC CCGCCACTTC CGGCTCCGAA ACTCCCGGCA CAAGCGAGAG
2451    CGCTACCCCA GAGTCAGGAC CAGGAACATC TACAGAGCCC TCTGAAGGCT
2501    CCGCTCCAGG GTCCCCAGCC GGCAGTCCCA CTAGCACCGA GGAGGGAACC
2551    TCTGAAAGCG CCACACCCGA ATCAGGGCCA GGGTCTGAGC CTGCTACCAG
2601    CGGCAGCGAG ACACCAGGCA CCTCTGAGTC CGCCACACCA GAGTCCGGAC
2651    CCGGATCTCC CGCTGGGAGC CCCACCTCCA CTGAGGAGGG ATCTCCTGCT
2701    GGCTCTCCAA CATCTACTGA GGAAGGTACC TCAACCGAGC CATCCGAGGG
2751    ATCAGCTCCC GGCACCTCAG AGTCGGCAAC CCCGGAGTCT GGACCCGGAA
2801    CTTCCGAAAG TGCCACACCA GAGTCCGGTC CCGGGACTTC AGAATCAGCA
2851    ACACCCGAGT CCGGCCCTGG GTCTGAACCC GCCACAAGTG GTAGTGAGAC
2901    ACCAGGATCA GAACCTGCTA CCTCAGGGTC AGAGACACCC GGATCTCCGG
2951    CAGGCTCACC AACCTCCACT GAGGAGGGCA CCAGCACAGA ACCAAGCGAG
3001    GGCTCCGCAC CCGGAACAAG CACTGAACCC AGTGAGGGTT CAGCACCCGG
3051    CTCTGAGCCG GCCACAAGTG GCAGTGAGAC ACCCGGCACT TCAGAGAGTG
3101    CCACCCCCGA GAGTGGCCCA GGCACTAGTA CCGAGCCCTC TGAAGGCAGT
3151    GCGCCAGCCT CGAGCGAAAT AACTCGTACT ACTCTTCAGT CAGATCAAGA
3201    GGAAATCGAT TATGATGATA CCATATCAGT TGAAATGAAG AAGGAAGATT
3251    TTGACATTTA TGATGAGGAT GAAAATCAGA GCCCCCGCAG CTTTCAAAAG
3301    AAAACACGAC ACTATTTTAT TGCTGCAGTG GAGAGGCTCT GGGATTATGG
3351    GATGAGTAGC TCCCCACATG TTCTAAGAAA CAGGGCTCAG AGTGGCAGTG
3401    TCCCTCAGTT CAAGAAAGTT GTTTTCCAGG AATTTACTGA TGGCTCCTTT
3451    ACTCAGCCCT TATACCGTGG AGAACTAAAT GAACATTTGG GACTCCTGGG
3501    GCCATATATA AGAGCAGAAG TTGAAGATAA TATCATGGTA ACTTTCAGAA
3551    ATCAGGCCTC TCGTCCCTAT TCCTTCTATT CTAGCCTTAT TTCTTATGAG
3601    GAAGATCAGA GGCAAGGAGC AGAACCTAGA AAAAACTTTG TCAAGCCTAA
3651    TGAAACCAAA ACTTACTTTT GGAAAGTGCA ACATCATATG GCACCCACTA
3701    AAGATGAGTT TGACTGCAAA GCCTGGGCTT ATTTCTCTGA TGTTGACCTG
3751    GAAAAAGATG TGCACTCAGG CCTGATTGGA CCCCTTCTGG TCTGCCACAC
3801    TAACACACTG AACCCTGCTC ATGGGAGACA AGTGACAGTA CAGGAATTTG
3851    CTCTGTTTTT CACCATCTTT GATGAGACCA AAAGCTGGTA CTTCACTGAA
3901    AATATGGAAA GAAACTGCAG GGCTCCCTGC AATATCCAGA TGGAAGATCC
3951    CACTTTTAAA GAGAATTATC GCTTCCATGC AATCAATGGC TACATAATGG
4001    ATACACTACC TGGCTTAGTA ATGGCTCAGG ATCAAAGGAT TCGATGGTAT
4051    CTGCTCAGCA TGGGCAGCAA TGAAAACATC CATTCTATTC ATTTCAGTGG
4101    ACATGTGTTC ACTGTACGAA AAAAAGAGGA GTATAAAATG GCACTGTACA
4151    ATCTCTATCC AGGTGTTTTT GAGACAGTGG AAATGTTACC ATCCAAAGCT
4201    GGAATTTGGC GGGTGGAATG CCTTATTGGC GAGCATCTAC ATGCTGGGAT
4251    GAGCACACTT TTTCTGGTGT ACAGCAATAA GTGTCAGACT CCCCTGGGAA
4301    TGGCTTCTGG ACACATTAGA GATTTTCAGA TTACAGCTTC AGGACAATAT
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4351    GGACAGTGGG CCCCAAAGCT GGCCAGACTT CATTATTCCG GATCAATCAA
4401    TGCCTGGAGC ACCAAGGAGC CCTTTTCTTG GATCAAGGTG GATCTGTTGG
4451    CACCAATGAT TATTCACGGC ATCAAGACCC AGGGTGCCCG TCAGAAGTTC
4501    TCCAGCCTCT ACATCTCTCA GTTTATCATC ATGTATAGTC TTGATGGGAA
4551    GAAGTGGCAG ACTTATCGAG GAAATTCCAC TGGAACCTTA ATGGTCTTCT
4601    TTGGCAATGT GGATTCATCT GGGATAAAAC ACAATATTTT TAACCCTCCA
4651    ATTATTGCTC GATACATCCG TTTGCACCCA ACTCATTATA GCATTCGCAG
4701    CACTCTTCGC ATGGAGTTGA TGGGCTGTGA TTTAAATAGT TGCAGCATGC
4751    CATTGGGAAT GGAGAGTAAA GCAATATCAG ATGCACAGAT TACTGCTTCA
4801    TCCTACTTTA CCAATATGTT TGCCACCTGG TCTCCTTCAA AAGCTCGACT
4851    TCACCTCCAA GGGAGGAGTA ATGCCTGGAG ACCTCAGGTG AATAATCCAA
4901    AAGAGTGGCT GCAAGTGGAC TTCCAGAAGA CAATGAAAGT CACAGGAGTA
4951    ACTACTCAGG GAGTAAAATC TCTGCTTACC AGCATGTATG TGAAGGAGTT
5001    CCTCATCTCC AGCAGTCAAG ATGGCCATCA GTGGACTCTC TTTTTTCAGA
5051    ATGGCAAAGT AAAGGTTTTT CAGGGAAATC AAGACTCCTT CACACCTGTG

5101    GTGAACTCTC TAGACCCACC GTTACTGACT CGCTACCTTC GAATTCACCC
5151    CCAGAGTTGG GTGCACCAGA TTGCCCTGAG GATGGAGGTT CTGGGCTGCG
5201    AGGCACAGGA CCTCTACGAC AAAACTCACA CATGCCCACC GTGCCCAGCT
5251    CCAGAACTCC TGGGCGGACC GTCAGTCTTC CTCTTCCCCC CAAAACCCAA
5301    GGACACCCTC ATGATCTCCC GGACCCCTGA GGTCACATGC GTGGTGGTGG
5351    ACGTGAGCCA CGAAGACCCT GAGGTCAAGT TCAACTGGTA CGTGGACGGC
5401    GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG
5451    CACGTACCGT GTGGTCAGCG TCCTCACCGT CCTGCACCAG GACTGGCTGA
5501    ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC
5551    ATCGAGAAAA CCATCTCCAA AGCCAAAGGG CAGCCCCGAG AACCACAGGT
5601    GTACACCCTG CCCCCATCCC GGGATGAGCT GACCAAGAAC CAGGTCAGCC
5651    TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC CGTGGAGTGG
5701    GAGAGCAATG GGCAGCCGGA GAACAACTAC AAGACCACGC CTCCCGTGTT
5751    GGACTCCGAC GGCTCCTTCT TCCTCTACAG CAAGCTCACC GTGGACAAGA
5801    GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT
5851    CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC CGGGTAAATG
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pSYN FVIII 312 protein sequence (FVIII with complete B-domain deletion except 5 amino acid residues and 288 AE-XTEN inserted after aa 745- B5 version) (SEQ ID NO:173)

```
   1    ATRRYYLGAV ELSWDYMQSD LGELPVDARF PPRVPKSFPF NTSVVYKKTL
  51    FVEFTDHLFN IAKPRPPWMG LLGPTIQAEV YDTVVITLKN MASHPVSLHA
 101    VGVSYWKASE GAEYDDQTSQ REKEDDKVFP GGSHTYVWQV LKENGPMASD
 151    PLCLTYSYLS HVDLVKDLNS GLIGALLVCR EGSLAKEKTQ TLHKFILLFA
 201    VFDEGKSWHS ETKNSLMQDR DAASARAWPK MHTVNGYVNR SLPGLIGCHR
 251    KSVYWHVIGM GTTPEVHSIF LEGHTFLVRN HRQASLEISP ITFLTAQTLL
 301    MDLGQFLLFC HISSHQHDGM EAYVKVDSCP EEPQLRMKNN EEAEDYDDDL
 351    TDSEMDVVRF DDDNSPSFIQ IRSVAKKHPK TWVHYIAAEE EDWDYAPLVL
 401    APDDRSYKSQ YLNNGPQRIG RKYKKVRFMA YTDETFKTRE AIQHESGILG
 451    PLLYGEVGDT LLIIFKNQAS RPYNIYPHGI TDVRPLYSRR LPKGVKHLKD
 501    FPILPGEIFK YKWTVTVEDG PTKSDPRCLT RYYSSFVNME RDLASGLIGP
 551    LLICYKESVD QRGNQIMSDK RNVILFSVFD ENRSWYLTEN IQRFLPNPAG
 601    VQLEDPEFQA SNIMHSINGY VFDSLQLSVC LHEVAYWYIL SIGAQTDFLS
 651    VFFSGYTFKH KMVYEDTLTL FPFSGETVFM SMENPGLWIL GCHNSDFRNR
 701    GMTALLKVSS CDKNTGDYYE DSYEDISAYL LSKNNAIEPR SFSQNGTSES
 751    ATPESGPGSE PATSGSETPG TSESATPESG PGSEPATSGS ETPGTSESAT
 801    PESGPGTSTE PSEGSAPGSP AGSPTSTEEG TSESATPESG PGSEPATSGS
 851    ETPGTSESAT PESGPGSPAG SPTSTEEGSP AGSPTSTEEG TSTEPSEGSA
 901    PGTSESATPE SGPGTSESAT PESGPGTSES ATPESGPGSE PATSGSETPG
 951    SEPATSGSET PGSPAGSPTS TEEGTSTEPS EGSAPGTSTE PSEGSAPGSE
1001    PATSGSETPG TSESATPESG PGTSTEPSEG SAPASSEITR TTLQSDQEEI
1051    DYDDTISVEM KKEDFDIYDE DENQSPRSFQ KKTRHYFIAA VERLWDYGMS
1101    SSPHVLRNRA QSGSVPQFKK VVFQEFTDGS FTQPLYRGEL NEHLGLLGPY
1151    IRAEVEDNIM VTFRNQASRP YSFYSSLISY EEDQRQGAEP RKNFVKPNET
1201    KTYFWKVQHH MAPTKDEFDC KAWAYFSDVD LEKDVHSGLI GPLLVCHTNT
1251    LNPAHGRQVT VQEFALFFTI FDETKSWYFT ENMERNCRAP CNIQMEDPTF
1301    KENYRFHAIN GYIMDTLPGL VMAQDQRIRW YLLSMGSNEN IHSIHFSGHV
1351    FTVRKKEEYK MALYNLYPGV FETVEMLPSK AGIWRVECLI GEHLHAGMST
1401    LFLVYSNKCQ TPLGMASGHI RDFQITASGQ YGQWAPKLAR LHYSGSINAW
1451    STKEPFSWIK VDLLAPMIIH GIKTQGARQK FSSLYISQFI IMYSLDGKKW
1501    QTYRGNSTGT LMVFFGNVDS SGIKHNIFNP PIIARYIRLH PTHYSIRSTL
1551    RMELMGCDLN SCSMPLGMES KAISDAQITA SSYFTNMFAT WSPSKARLHL
1601    QGRSNAWRPQ VNNPKEWLQV DFQKTMKVTG VTTQGVKSLL TSMYVKEFLI
1651    SSSQDGHQWT LFFQNGKVKV FQGNQDSFTP VVNSLDPPLL TRYLRIHPQS
1701    WVHQIALRME VLGCEAQDLY DKTHTCPPCP APELLGGPSV FLFPPKPKDT
1751    LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
1801    RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
1851    LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS

1901    DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK*
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pSYN VWF059 nucleotide sequence (encoding VWF D'D3-Fc with acidic region 2 (a2) thrombin site in the linker) (SEQ ID NO: 196)

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   1    ATGATTCCTG CCAGATTTGC CGGGGTGCTG CTTGCTCTGG CCCTCATTTT
  51    GCCAGGGACC CTTTGTGCAG AAGGAACTCG CGGCAGGTCA TCCACGGCCC
 101    GATGCAGCCT TTTCGGAAGT GACTTCGTCA ACACCTTTGA TGGGAGCATG
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 151    TACAGCTTTG CGGGATACTG CAGTTACCTC CTGGCAGGGG GCTGCCAGAA
 201    ACGCTCCTTC TCGATTATTG GGGACTTCCA GAATGGCAAG AGAGTGAGCC
 251    TCTCCGTGTA TCTTGGGGAA TTTTTTGACA TCCATTTGTT TGTCAATGGT
 301    ACCGTGACAC AGGGGGACCA AAGAGTCTCC ATGCCCTATG CCTCCAAAGG
 351    GCTGTATCTA GAAACTGAGG CTGGGTACTA CAAGCTGTCC GGTGAGGCCT
 401    ATGGCTTTGT GGCCAGGATC GATGGCAGCG GCAACTTTCA AGTCCTGCTG
 451    TCAGACAGAT ACTTCAACAA GACCTGCGGG CTGTGTGGCA ACTTTAACAT
 501    CTTTGCTGAA GATGACTTTA TGACCCAAGA AGGGACCTTG ACCTCGGACC
 551    CTTATGACTT TGCCAACTCA TGGGCTCTGA GCAGTGGAGA ACAGTGGTGT
 601    GAACGGGCAT CTCCTCCCAG CAGCTCATGC AACATCTCCT CTGGGGAAAT
 651    GCAGAAGGGC CTGTGGGAGC AGTGCCAGCT TCTGAAGAGC ACCTCGGTGT
 701    TTGCCCGCTG CCACCCTCTG GTGGACCCCG AGCCTTTTGT GGCCCTGTGT
 751    GAGAAGACTT TGTGTGAGTG TGCTGGGGGG CTGGAGTGCG CCTGCCCTGC
 801    CCTCCTGGAG TACGCCCGGA CCTGTGCCCA GGAGGGAATG GTGCTGTACG
 851    GCTGGACCGA CCACAGCGCG TGCAGCCCAG TGTGCCCTGC TGGTATGGAG
 901    TATAGGCAGT GTGTGTCCCC TTGCGCCAGG ACCTGCCAGA GCCTGCACAT
 951    CAATGAAATG TGTCAGGAGC GATGCGTGGA TGGCTGCAGC TGCCCTGAGG
1001    GACAGCTCCT GGATGAAGGC CTCTGCGTGG AGAGCACCGA GTGTCCCTGC
1051    GTGCATTCCG GAAAGCGCTA CCCTCCCGGC ACCTCCCTCT CTCGAGACTG
1101    CAACACCTGC ATTTGCCGAA ACAGCCAGTG GATCTGCAGC AATGAAGAAT
1151    GTCCAGGGGA GTGCCTTGTC ACTGGTCAAT CCCACTTCAA GAGCTTTGAC
1201    AACAGATACT TCACCTTCAG TGGGATCTGC CAGTACCTGC TGGCCCGGGA
1251    TTGCCAGGAC CACTCCTTCT CCATTGTCAT TGAGACTGTC CAGTGTGCTG
1301    ATGACCGCGA CGCTGTGTGC ACCCGCTCCG TCACCGTCCG GCTGCCTGGC
1351    CTGCACAACA GCCTTGTGAA ACTGAAGCAT GGGGCAGGAG TTGCCATGGA
1401    TGGCCAGGAC ATCCAGCTCC CCCTCCTGAA AGGTGACCTC CGCATCCAGC
1451    ATACAGTGAC GGCCTCCGTG CGCCTCAGCT ACGGGGAGGA CCTGCAGATG
1501    GACTGGGATG GCCGCGGGAG GCTGCTGGTG AAGCTGTCCC CCGTCTATGC
1551    CGGGAAGACC TGCGGCCTGT GTGGGAATTA CAATGGCAAC CAGGGCGACG
1601    ACTTCCTTAC CCCCTCTGGG CTGGCGGAGC CCCGGGTGGA GGACTTCGGG
1651    AACGCCTGGA AGCTGCACGG GGACTGCCAG GACCTGCAGA AGCAGCACAG
1701    CGATCCCTGC GCCCTCAACC CGCGCATGAC CAGGTTCTCC GAGGAGGCGT
1751    GCGCGGTCCT GACGTCCCCC ACATTCGAGG CCTGCCATCG TGCCGTCAGC
1801    CCGCTGCCCT ACCTGCGGAA CTGCCGCTAC GACGTGTGCT CCTGCTCGGA
1851    CGGCCGCGAG TGCCTGTGCG GCGCCCTGGC CAGCTATGCC GCGGCCTGCG
1901    CGGGGAGAGG CGTGCGCGTC GCGTGGCGCG AGCCAGGCCG CTGTGAGCTG
1951    AACTGCCCGA AAGGCCAGGT GTACCTGCAG TGCGGGACCC CCTGCAACCT
2001    GACCTGCCGC TCTCTCTCTT ACCCGGATGA GGAATGCAAT GAGGCCTGCC
2051    TGGAGGGCTG CTTCTGCCCC CCAGGGCTCT ACATGGATGA GAGGGGGGAC
2101    TGCGTGCCCA AGGCCCAGTG CCCCTGTTAC TATGACGGTG AGATCTTCCA
2151    GCCAGAAGAC ATCTTCTCAG ACCATCACAC CATGTGCTAC TGTGAGGATG
2201    GCTTCATGCA CTGTACCATG AGTGGAGTCC CCGGAAGCTT GCTGCCTGAC
2251    GCTGTCCTCA GCAGTCCCCT GTCTCATCGC AGCAAAAGGA GCCTATCCTG
2301    TCGGCCCCCC ATGGTCAAGC TGGTGTGTCC CGCTGACAAC CTGCGGGCTG
2351    AAGGGCTCGA GTGTACCAAA ACGTGCCAGA ACTATGACCT GGAGTGCATG
2401    AGCATGGGCT GTGTCTCTGG CTGCCTCTGC CCCCCGGGCA TGGTCCGGCA
2451    TGAGAACAGA TGTGTGGCCC TGGAAAGGTG TCCCTGCTTC CATCAGGGCA
2501    AGGAGTATGC CCCTGGAGAA ACAGTGAAGA TTGGCTGCAA CACTTGTGTC
2551    TGTCGGGACC GGAAGTGGAA CTGCACAGAC CATGTGTGTG ATGCCACGTG
2601    CTCCACGATC GGCATGGCCC ACTACCTCAC CTTCGACGGG CTCAAATACC

2651    TGTTCCCCGG GGAGTGCCAG TACGTTCTGG TGCAGGATTA CTGCGGCAGT
2701    AACCCTGGGA CCTTTCGGAT CCTAGTGGGG AATAAGGGAT GCAGCCACCC
2751    CTCAGTGAAA TGCAAGAAAC GGGTCACCAT CCTGGTGGAG GGAGGAGAGA
2801    TTGAGCTGTT TGACGGGGAG GTGAATGTGA AGAGGCCCAT GAAGGATGAG
2851    ACTCACTTTG AGGTGGTGGA GTCTGGCCGG TACATCATTC TGCTGCTGGG
2901    CAAAGCCCTC TCCGTGGTCT GGGACCGCCA CCTGAGCATC TCCGTGGTCC
2951    TGAAGCAGAC ATACCAGGAG AAAGTGTGTG GCCTGTGTGG GAATTTTGAT
3001    GGCATCCAGA ACAATGACCT CACCAGCAGC AACCTCCAAG TGGAGGAAGA
3051    CCCTGTGGAC TTTGGGAACT CCTGGAAAGT GAGCTCGCAG TGTGCTGACA
3101    CCAGAAAAGT GCCTCTGGAC TCATCCCCTG CCACCTGCCA TAACAACATC
3151    ATGAAGCAGA CGATGGTGGA TTCCTCCTGT AGAATCCTTA CCAGTGACGT
3201    CTTCCAGGAC TGCAACAAGC TGGTGGACCC CGAGCCATAT CTGGATGTCT
3251    GCATTTACGA CACCTGCTCC TGTGAGTCCA TTGGGGACTG CGCCGCATTC
3301    TGCGACACCA TTGCTGCCTA TGCCCACGTG TGTGCCCAGC ATGGCAAGGT
3351    GGTGACCTGG AGGACGGCCA CATTGTGCCC CCAGAGCTGC GAGGAGAGGA
3401    ATCTCCGGGA GAACGGGTAT GAGGCTGAGT GGCGCTATAA CAGCTGTGCA
3451    CCTGCCTGTC AAGTCACGTG TCAGCACCCT GAGCCACTGG CCTGCCCTGT
3501    GCAGTGTGTG GAGGGCTGCC ATGCCCACTG CCCTCCAGGG AAAATCCTGG
3551    ATGAGCTTTT GCAGACCTGC GTTGACCCTG AAGACTGTCC AGTGTGTGAG
3601    GTGGCTGGCC GGCGTTTTGC CTCAGGAAAG AAAGTCACCT TGAATCCCAG
3651    TGACCCTGAG CACTGCCAGA TTTGCCACTG TGATGTTGTC AACCTCACCT
3701    GTGAAGCCTG CCAGGAGCCG ATATCGGGCG CGCCAACATC AGAGAGCGCC
3751    ACCCCTGAAA GTGGTCCCGG GAGCGAGCCA GCCACATCTG GGTCGGAAAC
3801    GCCAGGCACA AGTGAGTCTG CAACTCCCGA GTCCGGACCT GGCTCCGAGC
3851    CTGCCACTAG CGGCTCCGAG ACTCCGGGAA CTTCCGAGAG CGCTACACCA
3901    GAAAGCGGAC CCGGAACCAG TACCGAACCT AGCGAGGGCT CTGCTCCGGG
3951    CAGCCCAGCC GGCTCTCCTA CATCCACGGA GGAGGGCACT TCCGAATCCG
4001    CCACCCCGGA GTCAGGGCCA GGATCTGAAC CCGCTACCTC AGGCAGTGAG
4051    ACGCCAGGAA CGAGCGAGTC CGCTACACCG GAGAGTGGGC CAGGGAGCCC
4101    TGCTGGATCT CCTACGTCCA CTGAGGAAGG GTCACCAGCG GGCTCGCCCA
4151    CCAGCACTGA AGAAGGTGCC TCGATATCTG ACAAGAACAC TGGTGATTAT
4201    TACGAGGACA GTTATGAAGA TATTTCAGCA TACTTGCTGA GTAAAAACAA
4251    TGCCATTGAA CCAAGAAGCT TCTCTGACAA AACTCACACA TGCCCACCGT
4301    GCCCAGCTCC AGAACTCCTG GGCGGACCGT CAGTCTTCCT CTTCCCCCCA
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4351    AAACCCAAGG ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT
4401    GGTGGTGGAC GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG
4451    TGGACGGCGT GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG
4501    TACAACAGCA CGTACCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA
4551    CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC
4601    CAGCCCCCAT CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA
4651    CCACAGGTGT ACACCCTGCC CCCATCCCGG GATGAGCTGA CCAAGAACCA
4701    GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG
4751    TGGAGTGGGA GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACGCCT
4801    CCCGTGTTGG ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT
4851    GGACAAGAGC AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC
4901    ATGAGGCTCT GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG
4951    GGTAAATGA
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pSYN VWF059 protein sequence (VWF D'D3-Fc with a2 region of FVIII thrombin site in the linker) - bold underlined area shows a2 region (SEQ ID NO: 197)

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  1     MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFVNTFDGSM
 51     YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
101     TVTQGDQRVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
151     SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
201     ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
251     EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
301     YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTECPC
351     VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD

401     NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG
451     LHNSLVKLKH GAGVAMDGQD IQLPLLKGDL RIQHTVTASV RLSYGEDLQM
501     DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
551     NAWKLHGDCQ DLQKQHSDPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
601     PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCEL
651     NCPKGQVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP PGLYMDERGD
701     CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
751     AVLSSPLSHR SKRSLSCRPP MVKLVCPADN LRAEGLECTK TCQNYDLECM
801     SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
851     CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGECQ YVLVQDYCGS
901     NPGTFRILVG NKGCSHPSVK CKKRVTILVE GGEIELFDGE VNVKRPMKDE
951     THFEVVESGR YIILLLGKAL SVVWDRHLSI SVVLKQTYQE KVCGLCGNFD
1001    GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
1051    MKQTMVDSSC RILTSDVFQD CNKLVDPEPY LDVCIYDTCS CESIGDCAAF
1101    CDTIAAYAHV CAQHGKVVTW RTATLCPQSC EERNLRENGY EAEWRYNSCA
1151    PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
1201    VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP ISGAPTSESA
1251    TPESGPGSEP ATSGSETPGT SESATPESGP GSEPATSGSE TPGTSESATP
1301    ESGPGTSTEP SEGSAPGSPA GSPTSTEEGT SESATPESGP GSEPATSGSE
1351    TPGTSESATP ESGPGSPAGS PTSTEEGSPA GSPTSTEEGA SISDKNTGDY
1401    YEDSYEDISA YLLSKNNAIE PRSFSDKTHT CPPCPAPELL GGPSVFLFPP
1451    KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
1501    YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
1551    PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP
1601    PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
1651    GK*
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pSYN VWF062 nucleotide sequence (encoding VWF D'D3-Fc with no thrombin site in the linker) (SEQ ID NO: 198)

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  1     ATGATTCCTG CCAGATTTGC CGGGGTGCTG CTTGCTCTGG CCCTCATTTT
 51     GCCAGGGACC CTTTGTGCAG AAGGAACTCG CGGCAGGTCA TCCACGGCCC
101     GATGCAGCCT TTTCGGAAGT GACTTCGTCA ACACCTTTGA TGGGAGCATG
151     TACAGCTTTG CGGGATACTG CAGTTACCTC CTGGCAGGGG GCTGCCAGAA
201     ACGCTCCTTC TCGATTATTG GGGACTTCCA GAATGGCAAG AGAGTGAGCC
251     TCTCCGTGTA TCTTGGGGAA TTTTTTGACA TCCATTTGTT TGTCAATGGT
301     ACCGTGACAC AGGGGGACCA AAGAGTCTCC ATGCCCTATG CCTCCAAAGG
351     GCTGTATCTA GAAACTGAGG CTGGGTACTA CAAGCTGTCC GGTGAGGCCT
401     ATGGCTTTGT GGCCAGGATC GATGGCAGCG GCAACTTTCA AGTCCTGCTG
451     TCAGACAGAT ACTTCAACAA GACCTGCGGG CTGTGTGGCA ACTTTAACAT
501     CTTTGCTGAA GATGACTTTA TGACCCAAGA AGGGACCTTG ACCTCGGACC
551     CTTATGACTT TGCCAACTCA TGGGCTCTGA GCAGTGGAGA ACAGTGGTGT
601     GAACGGGCAT CTCCTCCCAG CAGCTCATGC AACATCTCCT CTGGGGAAAT
651     GCAGAAGGGC CTGTGGGAGC AGTGCCAGCT TCTGAAGAGC ACCTCGGTGT
701     TTGCCCGCTG CCACCCTCTG GTGGACCCCG AGCCTTTTGT GGCCCTGTGT
751     GAGAAGACTT TGTGTGAGTG TGCTGGGGGG CTGGAGTGCG CCTGCCCTGC
801     CCTCCTGGAG TACGCCCGGA CCTGTGCCCA GGAGGGAATG GTGCTGTACG
851     GCTGGACCGA CCACAGCGCG TGCAGCCCAG TGTGCCCTGC TGGTATGGAG
901     TATAGGCAGT GTGTGTCCCC TTGCGCCAGG ACCTGCCAGA GCCTGCACAT
951     CAATGAAATG TGTCAGGAGC GATGCGTGGA TGGCTGCAGC TGCCCTGAGG
1001    GACAGCTCCT GGATGAAGGC CTCTGCGTGG AGAGCACCGA GTGTCCCTGC
1051    GTGCATTCCG GAAAGCGCTA CCCTCCCGGC ACCTCCCTCT CTCGAGACTG
1101    CAACACCTGC ATTTGCCGAA ACAGCCAGTG GATCTGCAGC AATGAAGAAT
1151    GTCCAGGGGA GTGCCTTGTC ACTGGTCAAT CCCACTTCAA GAGCTTTGAC
1201    AACAGATACT TCACCTTCAG TGGGATCTGC CAGTACCTGC TGGCCCGGGA
1251    TTGCCAGGAC CACTCCTTCT CCATTGTCAT TGAGACTGTC CAGTGTGCTG
1301    ATGACCGCGA CGCTGTGTGC ACCCGCTCCG TCACCGTCCG GCTGCCTGGC
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1351    CTGCACAACA GCCTTGTGAA ACTGAAGCAT GGGGCAGGAG TTGCCATGGA
1401    TGGCCAGGAC ATCCAGCTCC CCCTCCTGAA AGGTGACCTC CGCATCCAGC

1451    ATACAGTGAC GGCCTCCGTG CGCCTCAGCT ACGGGGAGGA CCTGCAGATG
1501    GACTGGGATG GCCGCGGGAG GCTGCTGGTG AAGCTGTCCC CCGTCTATGC
1551    CGGGAAGACC TGCGGCCTGT GTGGGAATTA CAATGGCAAC CAGGGCGACG
1601    ACTTCCTTAC CCCCTCTGGG CTGGCGGAGC CCCGGGTGGA GGACTTCGGG
1651    AACGCCTGGA AGCTGCACGG GGACTGCCAG GACCTGCAGA AGCAGCACAG
1701    CGATCCCTGC GCCCTCAACC CGCGCATGAC CAGGTTCTCC GAGGAGGCGT
1751    GCGCGGTCCT GACGTCCCCC ACATTCGAGG CCTGCCATCG TGCCGTCAGC
1801    CCGCTGCCCT ACCTGCGGAA CTGCCGCTAC GACGTGTGCT CCTGCTCGGA
1851    CGGCCGCGAG TGCCTGTGCG GCGCCCTGGC CAGCTATGCC GCGGCCTGCG
1901    CGGGGAGAGG CGTGCGCGTC GCGTGGCGCG AGCCAGGCCG CTGTGAGCTG
1951    AACTGCCCGA AAGGCCAGGT GTACCTGCAG TGCGGGACCC CCTGCAACCT
2001    GACCTGCCGC TCTCTCTCTT ACCCGGATGA GGAATGCAAT GAGGCCTGCC
2051    TGGAGGGCTG CTTCTGCCCC CCAGGGCTCT ACATGGATGA GAGGGGGGAC
2101    TGCGTGCCCA AGGCCCAGTG CCCCTGTTAC TATGACGGTG AGATCTTCCA
2151    GCCAGAAGAC ATCTTCTCAG ACCATCACAC CATGTGCTAC TGTGAGGATG
2201    GCTTCATGCA CTGTACCATG AGTGGAGTCC CCGGAAGCTT GCTGCCTGAC
2251    GCTGTCCTCA GCAGTCCCCT GTCTCATCGC AGCAAAAGGA GCCTATCCTG
2301    TCGGCCCCCC ATGGTCAAGC TGGTGTGTCC CGCTGACAAC CTGCGGGCTG
2351    AAGGGCTCGA GTGTACCAAA ACGTGCCAGA ACTATGACCT GGAGTGCATG
2401    AGCATGGGCT GTGTCTCTGG CTGCCTCTGC CCCCCGGGCA TGGTCCGGCA
2451    TGAGAACAGA TGTGTGGCCC TGGAAAGGTG TCCCTGCTTC CATCAGGGCA
2501    AGGAGTATGC CCCTGGAGAA ACAGTGAAGA TTGGCTGCAA CACTTGTGTC
2551    TGTCGGGACC GGAAGTGGAA CTGCACAGAC CATGTGTGTG ATGCCACGTG
2601    CTCCACGATC GGCATGGCCC ACTACCTCAC CTTCGACGGG CTCAAATACC
2651    TGTTCCCCGG GGAGTGCCAG TACGTTCTGG TGCAGGATTA CTGCGGCAGT
2701    AACCCTGGGA CCTTTCGGAT CCTAGTGGGG AATAAGGGAT GCAGCCACCC
2751    CTCAGTGAAA TGCAAGAAAC GGGTCACCAT CCTGGTGGAG GGAGGAGAGA
2801    TTGAGCTGTT TGACGGGGAG GTGAATGTGA AGAGGCCCAT GAAGGATGAG
2851    ACTCACTTTG AGGTGGTGGA GTCTGGCCGG TACATCATTC TGCTGCTGGG
2901    CAAAGCCCTC TCCGTGGTCT GGGACCGCCA CCTGAGCATC TCCGTGGTCC
2951    TGAAGCAGAC ATACCAGGAG AAAGTGTGTG GCCTGTGTGG GAATTTTGAT
3001    GGCATCCAGA ACAATGACCT CACCAGCAGC AACCTCCAAG TGGAGGAAGA
3051    CCCTGTGGAC TTTGGGAACT CCTGGAAAGT GAGCTCGCAG TGTGCTGACA
3101    CCAGAAAAGT GCCTCTGGAC TCATCCCCTG CCACCTGCCA TAACAACATC
3151    ATGAAGCAGA CGATGGTGGA TTCCTCCTGT AGAATCCTTA CCAGTGACGT
3201    CTTCCAGGAC TGCAACAAGC TGGTGGACCC CGAGCCATAT CTGGATGTCT
3251    GCATTTACGA CACCTGCTCC TGTGAGTCCA TTGGGGACTG CGCCGCATTC
3301    TGCGACACCA TTGCTGCCTA TGCCCACGTG TGTGCCCAGC ATGGCAAGGT
3351    GGTGACCTGG AGGACGGCCA CATTGTGCCC CCAGAGCTGC GAGGAGAGGA
3401    ATCTCCGGGA GAACGGGTAT GAGGCTGAGT GGCGCTATAA CAGCTGTGCA
3451    CCTGCCTGTC AAGTCACGTG TCAGCACCCT GAGCCACTGG CCTGCCCTGT
3501    GCAGTGTGTG GAGGGCTGCC ATGCCCACTG CCCTCCAGGG AAAATCCTGG
3551    ATGAGCTTTT GCAGACCTGC GTTGACCCTG AAGACTGTCC AGTGTGTGAG
3601    GTGGCTGGCC GGCGTTTTGC CTCAGGAAAG AAAGTCACCT TGAATCCCAG
3651    TGACCCTGAG CACTGCCAGA TTTGCCACTG TGATGTTGTC AACCTCACCT
3701    GTGAAGCCTG CCAGGAGCCG ATATCGGGCG CGCCAACATC AGAGAGCGCC
3751    ACCCCTGAAA GTGGTCCCGG GAGCGAGCCA GCCACATCTG GGTCGGAAAC
3801    GCCAGGCACA AGTGAGTCTG CAACTCCCGA GTCCGGACCT GGCTCCGAGC
3851    CTGCCACTAG CGGCTCCGAG ACTCCGGGAA CTTCCGAGAG CGCTACACCA
3901    GAAAGCGGAC CCGGAACCAG TACCGAACCT AGCGAGGGCT CTGCTCCGGG
3951    CAGCCCAGCC GGCTCTCCTA CATCCACGGA GGAGGGCACT TCCGAATCCG
4001    CCACCCCGGA GTCAGGGCCA GGATCTGAAC CCGCTACCTC AGGCAGTGAG
4051    ACGCCAGGAA CGAGCGAGTC CGCTACACCG GAGAGTGGGC CAGGGAGCCC
4101    TGCTGGATCT CCTACGTCCA CTGAGGAAGG GTCACCAGCG GGCTCGCCCA
4151    CCAGCACTGA AGAAGGTGCC TCGAGCGACA AAACTCACAC ATGCCCACCG
4201    TGCCCAGCTC CAGAACTCCT GGGCGGACCG TCAGTCTTCC TCTTCCCCCC
4251    AAAACCCAAG GACACCCTCA TGATCTCCCG GACCCCTGAG GTCACATGCG
4301    TGGTGGTGGA CGTGAGCCAC GAAGACCCTG AGGTCAAGTT CAACTGGTAC
4351    GTGGACGGCG TGGAGGTGCA TAATGCCAAG ACAAAGCCGC GGGAGGAGCA

4401    GTACAACAGC ACGTACCGTG TGGTCAGCGT CCTCACCGTC CTGCACCAGG
4451    ACTGGCTGAA TGGCAAGGAG TACAAGTGCA AGGTCTCCAA CAAAGCCCTC
4501    CCAGCCCCCA TCGAGAAAAC CATCTCCAAA GCCAAAGGGC AGCCCCGAGA
4551    ACCACAGGTG TACACCCTGC CCCCATCCCG GGATGAGCTG ACCAAGAACC
4601    AGGTCAGCCT GACCTGCCTG GTCAAAGGCT TCTATCCCAG CGACATCGCC
4651    GTGGAGTGGG AGAGCAATGG GCAGCCGGAG AACAACTACA AGACCACGCC
4701    TCCCGTGTTG GACTCCGACG GCTCCTTCTT CCTCTACAGC AAGCTCACCG
4751    TGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTTCTCATG CTCCGTGATG
4801    CATGAGGCTC TGCACAACCA CTACACGCAG AAGAGCCTCT CCCTGTCTCC
4851    GGGTAAATGA
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pSYN VWF062 protein sequence (VWF D'D3-Fc with no thrombin site in the linker) (SEQ ID NO: 199)

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  51    YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
 101    TVTQGDQRVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
 151    SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
 201    ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
 251    EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
 301    YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTECPC
 351    VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD
 401    NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG
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 451    LHNSLVKLKH GAGVAMDGQD IQLPLLKGDL RIQHTVTASV RLSYGEDLQM
 501    DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
 551    NAWKLHGDCQ DLQKQHSDPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
 601    PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCEL
 651    NCPKGQVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP PGLYMDERGD
 701    CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
 751    AVLSSPLSHR SKRSLSCRPP MVKLVCPADN LRAEGLECTK TCQNYDLECM
 801    SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
 851    CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGECQ YVLVQDYCGS
 901    NPGTFRILVG NKGCSHPSVK CKKRVTILVE GGEIELFDGE VNVKRPMKDE
 951    THFEVVESGR YIILLLGKAL SVVWDRHLSI SVVLKQTYQE KVCGLCGNFD
1001    GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
1051    MKQTMVDSSC RILTSDVFQD CNKLVDPEPY LDVCIYDTCS CESIGDCAAF
1101    CDTIAAYAHV CAQHGKVVTW RTATLCPQSC EERNLRENGY EAEWRYNSCA
1151    PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
1201    VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP ISGAPTSESA
1251    TPESGPGSEP ATSGSETPGT SESATPESGP GSEPATSGSE TPGTSESATP
1301    ESGPGTSTEP SEGSAPGSPA GSPTSTEEGT SESATPESGP GSEPATSGSE
1351    TPGTSESATP ESGPGSPAGS PTSTEEGSPA GSPTSTEEGA SSDKTHTCPP
1401    CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY
1451    VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL
1501    PAPIEKTISK AKGQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA
1551    VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
1601    HEALHNHYTQ KSLSLSPGK*
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pSYN VWF073 nucleotide sequence- (encoding VWFD1D2D'D3- 144 AE XTEN- FVIII truncated a2 thrombin site-Fc) (SEQ ID NO:174)

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   1    ATGATTCCTG CCAGATTTGC CGGGGTGCTG CTTGCTCTGG CCCTCATTTT
  51    GCCAGGGACC CTTTGTGCAG AAGGAACTCG CGGCAGGTCA TCCACGGCCC
 101    GATGCAGCCT TTTCGGAAGT GACTTCGTCA ACACCTTTGA TGGGAGCATG
 151    TACAGCTTTG CGGGATACTG CAGTTACCTC CTGGCAGGGG GCTGCCAGAA

 201    ACGCTCCTTC TCGATTATTG GGGACTTCCA GAATGGCAAG AGAGTGAGCC
 251    TCTCCGTGTA TCTTGGGGAA TTTTTTGACA TCCATTTGTT TGTCAATGGT
 301    ACCGTGACAC AGGGGGACCA AAGAGTCTCC ATGCCCTATG CCTCCAAAGG
 351    GCTGTATCTA GAAACTGAGG CTGGGTACTA CAAGCTGTCC GGTGAGGCCT
 401    ATGGCTTTGT GGCCAGGATC GATGGCAGCG GCAACTTTCA AGTCCTGCTG
 451    TCAGACAGAT ACTTCAACAA GACCTGCGGG CTGTGTGGCA ACTTTAACAT
 501    CTTTGCTGAA GATGACTTTA TGACCCAAGA AGGGACCTTG ACCTCGGACC
 551    CTTATGACTT TGCCAACTCA TGGGCTCTGA GCAGTGGAGA ACAGTGGTGT
 601    GAACGGGCAT CTCCTCCCAG CAGCTCATGC AACATCTCCT CTGGGGAAAT
 651    GCAGAAGGGC CTGTGGGAGC AGTGCCAGCT TCTGAAGAGC ACCTCGGTGT
 701    TTGCCCGCTG CCACCCTCTG GTGGACCCCG AGCCTTTTGT GGCCCTGTGT
 751    GAGAAGACTT TGTGTGAGTG TGCTGGGGGG CTGGAGTGCG CCTGCCCTGC
 801    CCTCCTGGAG TACGCCCGGA CCTGTGCCCA GGAGGGAATG GTGCTGTACG
 851    GCTGGACCGA CCACAGCGCG TGCAGCCCAG TGTGCCCTGC TGGTATGGAG
 901    TATAGGCAGT GTGTGTCCCC TTGCGCCAGG ACCTGCCAGA GCCTGCACAT
 951    CAATGAAATG TGTCAGGAGC GATGCGTGGA TGGCTGCAGC TGCCCTGAGG
1001    GACAGCTCCT GGATGAAGGC CTCTGCGTGG AGAGCACCGA GTGTCCCTGC
1051    GTGCATTCCG GAAAGCGCTA CCCTCCCGGC ACCTCCCTCT CTCGAGACTG
1101    CAACACCTGC ATTTGCCGAA ACAGCCAGTG GATCTGCAGC AATGAAGAAT
1151    GTCCAGGGGA GTGCCTTGTC ACTGGTCAAT CCCACTTCAA GAGCTTTGAC
1201    AACAGATACT TCACCTTCAG TGGGATCTGC CAGTACCTGC TGGCCCGGGA
1251    TTGCCAGGAC CACTCCTTCT CCATTGTCAT TGAGACTGTC CAGTGTGCTG
1301    ATGACCGCGA CGCTGTGTGC ACCCGCTCCG TCACCGTCCG GCTGCCTGGC
1351    CTGCACAACA GCCTTGTGAA ACTGAAGCAT GGGGCAGGAG TTGCCATGGA
1401    TGGCCAGGAC ATCCAGCTCC CCCTCCTGAA AGGTGACCTC CGCATCCAGC
1451    ATACAGTGAC GGCCTCCGTG CGCCTCAGCT ACGGGGAGGA CCTGCAGATG
1501    GACTGGGATG GCCGCGGGAG GCTGCTGGTG AAGCTGTCCC CCGTCTATGC
1551    CGGGAAGACC TGCGGCCTGT GTGGGAATTA CAATGGCAAC CAGGGCGACG
1601    ACTTCCTTAC CCCCTCTGGG CTGGCGGAGC CCCGGGTGGA GGACTTCGGG
1651    AACGCCTGGA AGCTGCACGG GGACTGCCAG GACCTGCAGA AGCAGCACAG
1701    CGATCCCTGC GCCCTCAACC CGCGCATGAC CAGGTTCTCC GAGGAGGCGT
1751    GCGCGGTCCT GACGTCCCCC ACATTCGAGG CCTGCCATCG TGCCGTCAGC
1801    CCGCTGCCCT ACCTGCGGAA CTGCCGCTAC GACGTGTGCT CCTGCTCGGA
1851    CGGCCGCGAG TGCCTGTGCG GCGCCCTGGC CAGCTATGCC GCGGCCTGCG
1901    CGGGGAGAGG CGTGCGCGTC GCGTGGCGCG AGCCAGGCCG CTGTGAGCTG
1951    AACTGCCCGA AAGGCCAGGT GTACCTGCAG TGCGGGACCC CCTGCAACCT
2001    GACCTGCCGC TCTCTCTCTT ACCCGGATGA GGAATGCAAT GAGGCCTGCC
2051    TGGAGGGCTG CTTCTGCCCC CCAGGGCTCT ACATGGATGA GAGGGGGGAC
2101    TGCGTGCCCA AGGCCCAGTG CCCCTGTTAC TATGACGGTG AGATCTTCCA
2151    GCCAGAAGAC ATCTTCTCAG ACCATCACAC CATGTGCTAC TGTGAGGATG
2201    GCTTCATGCA CTGTACCATG AGTGGAGTCC CCGGAAGCTT GCTGCCTGAC
2251    GCTGTCCTCA GCAGTCCCCT GTCTCATCGC AGCAAAAGGA GCCTATCCTG
2301    TCGGCCCCCC ATGGTCAAGC TGGTGTGTCC CGCTGACAAC CTGCGGGCTG
2351    AAGGGCTCGA GTGTACCAAA ACGTGCCAGA ACTATGACCT GGAGTGCATG
2401    AGCATGGGCT GTGTCTCTGG CTGCCTCTGC CCCCCGGGCA TGGTCCGGCA
2451    TGAGAACAGA TGTGTGGCCC TGGAAAGGTG TCCCTGCTTC CATCAGGGCA
2501    AGGAGTATGC CCCTGGAGAA ACAGTGAAGA TTGGCTGCAA CACTTGTGTC
2551    TGTCGGGACC GGAAGTGGAA CTGCACAGAC CATGTGTGTG ATGCCACGTG
2601    CTCCACGATC GGCATGGCCC ACTACCTCAC CTTCGACGGG CTCAAATACC
2651    TGTTCCCCGG GGAGTGCCAG TACGTTCTGG TGCAGGATTA CTGCGGCAGT
2701    AACCCTGGGA CCTTTCGGAT CCTAGTGGGG AATAAGGGAT GCAGCCACCC
2751    CTCAGTGAAA TGCAAGAAAC GGGTCACCAT CCTGGTGGAG GGAGGAGAGA
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2751    CTCAGTGAAA TGCAAGAAAC GGGTCACCAT CCTGGTGGAG GGAGGAGAGA
2801    TTGAGCTGTT TGACGGGGAG GTGAATGTGA AGAGGCCCAT GAAGGATGAG
2851    ACTCACTTTG AGGTGGTGGA GTCTGGCCGG TACATCATTC TGCTGCTGGG
2901    CAAAGCCCTC TCCGTGGTCT GGGACCGCCA CCTGAGCATC TCCGTGGTCC
2951    TGAAGCAGAC ATACCAGGAG AAAGTGTGTG GCCTGTGTGG GAATTTTGAT
3001    GGCATCCAGA ACAATGACCT CACCAGCAGC AACCTCCAAG TGGAGGAAGA
3051    CCCTGTGGAC TTTGGGAACT CCTGGAAAGT GAGCTCGCAG TGTGCTGACA
3101    CCAGAAAAGT GCCTCTGGAC TCATCCCCTG CCACCTGCCA TAACAACATC

3151    ATGAAGCAGA CGATGGTGGA TTCCTCCTGT AGAATCCTTA CCAGTGACGT
3201    CTTCCAGGAC TGCAACAAGC TGGTGGACCC CGAGCCATAT CTGGATGTCT
3251    GCATTTACGA CACCTGCTCC TGTGAGTCCA TTGGGGACTG CGCCGCATTC
3301    TGCGACACCA TTGCTGCCTA TGCCCACGTG TGTGCCCAGC ATGGCAAGGT
3351    GGTGACCTGG AGGACGGCCA CATTGTGCCC CCAGAGCTGC GAGGAGAGGA
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3501    GCAGTGTGTG GAGGGCTGCC ATGCCCACTG CCCTCCAGGG AAAATCCTGG
3551    ATGAGCTTTT GCAGACCTGC GTTGACCCTG AAGACTGTCC AGTGTGTGAG
3601    GTGGCTGGCC GGCGTTTTGC CTCAGGAAAG AAAGTCACCT TGAATCCCAG
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3751    GAAAGTGGTC CCGGGAGCGA GCCAGCCACA TCTGGGTCGG AAACGCCAGG
3801    CACAAGTGAG TCTGCAACTC CCGAGTCCGG ACCTGGCTCC GAGCCTGCCA
3851    CTAGCGGCTC CGAGACTCCG GGAACTTCCG AGAGCGCTAC ACCAGAAAGC
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3951    AGCCGGCTCT CCTACATCCA CGGAGGAGGG CACTTCCGAA TCCGCCACCC
4001    CGGAGTCAGG GCCAGGATCT GAACCCGCTA CCTCAGGCAG TGAGACGCCA
4051    GGAACGAGCG AGTCCGCTAC ACCGGAGAGT GGGCCAGGGA GCCCTGCTGG
4101    ATCTCCTACG TCCACTGAGG AAGGGTCACC AGCGGGCTCG CCCACCAGCA
4151    CTGAAGAAGG TGCCTCGAGC GGCGGTGGAG GATCCGGTGG CGGGGGATCC
4201    GGTGGCGGGG GATCCGGTGG CGGGGGATCC GGTGGCGGGG GATCCGGTGG
4251    CGGGGGATCC ATTGAACCAA GAAGCTTCTC TGGCAGCGGA GGCGACAAAA
4301    CTCACACATG CCCACCGTGC CCAGCTCCAG AACTCCTGGG CGGACCGTCA
4351    GTCTTCCTCT TCCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC
4401    CCCTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCCTGAGG
4451    TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA
4501    AAGCCGCGGG AGGAGCAGTA CAACAGCACG TACCGTGTGG TCAGCGTCCT
4551    CACCGTCCTG CACCAGGACT GGCTGAATGG CAAGGAGTAC AAGTGCAAGG
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4651    AAAGGGCAGC CCCGAGAACC ACAGGTGTAC ACCCTGCCCC CATCCCGGGA
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4751    ATCCCAGCGA CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC
4801    AACTACAAGA CCACGCCTCC CGTGTTGGAC TCCGACGGCT CCTTCTTCCT
4851    CTACAGCAAG CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAACGTCT
4901    TCTCATGCTC CGTGATGCAT GAGGCTCTGC ACAACCACTA CACGCAGAAG
4951    AGCCTCTCCC TGTCTCCGGG TAAATGA
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pSYN VWF073 protein sequence- (VWFD1D2D'D3- 144 AE XTEN- truncated a2 thrombin site-Fc) (SEQ ID NO: 175)

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   1    MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFVNTFDGSM
  51    YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
 101    TVTQGDQRVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
 151    SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
 201    ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
 251    EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
 301    YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTECPC
 351    VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD
 401    NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG
 451    LHNSLVKLKH GAGVAMDGQD IQLPLLKGDL RIQHTVTASV RLSYGEDLQM
 501    DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
 551    NAWKLHGDCQ DLQKQHSDPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
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 651    NCPKGQVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP PGLYMDERGD
 701    CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
 751    AVLSSPLSHR SKRSLSCRPP MVKLVCPADN LRAEGLECTK TCQNYDLECM

 801     SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
 851     CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGECQ YVLVQDYCGS
 901     NPGTFRILVG NKGCSHPSVK CKKRVTILVE GGEIELFDGE VNVKRPMKDE
 951     THFEVVESGR YIILLLGKAL SVVWDRHLSI SVVLKQTYQE KVCGLCGNFD
1001     GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
1051     MKQTMVDSSC RILTSDVFQD CNKLVDPEPY LDVCIYDTCS CESIGDCAAF
1101     CDTIAAYAHV CAQHGKVVTW RTATLCPQSC EERNLRENGY EAEWRYNSCA
1151     PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
1201     VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP GAPTSESATP
1251     ESGPGSEPAT SGSETPGTSE SATPESGPGS EPATSGSETP GTSESATPES
1301     GPGTSTEPSE GSAPGSPAGS PTSTEEGTSE SATPESGPGS EPATSGSETP
1351     GTSESATPES GPGSPAGSPT STEEGSPAGS PTSTEEGASS GGGGSGGGGS
1401     GGGGSGGGGS GGGGSGGGGS IEPRSFSGSG GDKTHTCPPC PAPELLGGPS
1451     VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT
1501     KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
1551     KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN
1601     NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK
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1651 SLSLSPGK*

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Patentkrav

1. Kimært protein, som omfatter
(i) et første polypeptid, der omfatter
(a) et faktor VIII ("FVIII")-protein,
(b) en XTEN af det første polypeptid og
(c) en første Fc-region,
hvor FVIII-proteinet har en FVIII-aktivitet; og
(ii) et andet polypeptid, der omfatter
(a) et von Willebrand-faktor ("VWF")-fragment, der omfatter en aminosyresekvens, der er mindst 99 % identisk med aminosyresekvensen som vist ved aminosyrerne 764 til 1240 af SEQ ID NO: 21,
(b) en XTEN af det andet polypeptid,
(c) en 10-50 aminosyrer lang spaltbar linker, der omfatter en a2-region af FVIII, der omfatter aminosyresekvensen fra Glu720 til Arg740 af FVIII svarende til SEQ ID NO: 65, hvor a2-regionen kan spaltes af thrombin, og
(d) en anden Fc-region,
hvor VWF-fragmentet binder til FVIII-proteinet;
hvor XTEN'en af det andet polypeptid omfatter aminosyresekvensen ifølge SEQ ID NO: 58;
hvor XTEN'en af det første polypeptid omfatter aminosyresekvensen ifølge SEQ ID NO: 8;
hvor den første Fc-region er forbundet med den anden Fc-region via en disulfidbinding; og
hvor halveringstiden for det kimære protein er forlænget sammenlignet med et FVIII-protein uden VWF-fragmentet.

2. Kimært protein, som omfatter
(i) et første polypeptid, der omfatter
(a) et faktor VIII ("FVIII")-protein,
(b) en XTEN af det første polypeptid og
(c) en første Fc-region,
hvor FVIII-proteinet har en FVIII-aktivitet; og
(ii) et andet polypeptid, der omfatter
(a) et von Willebrand-faktor ("VWF")-fragment, der omfatter en aminosyresekvens, der er mindst 99 % identisk med

aminosyresekvensen som vist ved aminosyrerne 764 til 1240 af SEQ ID NO: 21,
(b) en XTEN af det andet polypeptid,
(c) en 10-50 aminosyrer lang spaltbar linker, der omfatter en a2-region af FVIII, der omfatter aminosyresekvensen fra Glu720 til Arg740 af FVIII svarende til SEQ ID NO: 65, hvor a2-regionen kan spaltes af thrombin, og
(d) en anden Fc-region,
hvor VWF-fragmentet binder til FVIII-proteinet,
hvor VWF-fragmentet indeholder en anden aminosyre end cystein, der er indsat ved substitution i stedet for en rest svarende til resterne 1099 og 1142 af SEQ ID NO: 21;
hvor XTEN'en af det andet polypeptid omfatter aminosyresekvensen ifølge SEQ ID NO: 58;
hvor XTEN'en af det første polypeptid omfatter aminosyresekvensen ifølge SEQ ID NO: 8;
hvor den første Fc-region er forbundet med den anden Fc-region via en disulfidbinding; og
hvor halveringstiden af det kimære protein er forlænget sammenlignet med et FVIII-protein uden VWF-fragmentet.

3. Kimært protein ifølge krav 1 eller 2, hvor FVIII-proteinet er en dobbeltkædet FVIII-isoform.

4. Kimært protein ifølge krav 3, hvor den dobbeltkædede FVIII-isoform omfatter en første kæde, der omfatter en tung kæde af FVIII, og en anden kæde, der omfatter en let kæde af FVIII, hvor den tunge kæde og den lette kæde er forbundet med hinanden via en metalbinding.

5. Kimært protein ifølge krav 1 eller 2, hvor FVIII-proteinet er en enkeltkædet FVIII-isoform.

6. Kimært protein ifølge et hvilket som helst af kravene 1, 2 eller 5, hvor FVIII-proteinet har en aminosyresekvens, der er mindst 99 % identisk med aminosyrerne 1 til 1438 af SEQ ID NO: 67, og hvor FVIII-proteinet har koaguleringsaktivitet.

7. Kimært protein ifølge krav 6, hvor XTEN'en af det første polypeptid er indsat umiddelbart nedstrøms for resten svarende til rest 745 af SEQ ID NO: 67.

8. Kimært protein ifølge et hvilket som helst af kravene 1-7, hvor det andet polypeptid omfatter et D'-domæne og et D3-domæne af VWF, der er fusioneret til den anden Fc-region via XTEN'en af det andet polypeptid derimellem,
hvor XTEN-sekvensen indeholder mindre end 288 aminosyrerester, og
hvor XTEN'en af det andet polypeptid er fusioneret til den anden Fc-region via den spaltbare linker.

9. Kimært protein ifølge krav 2, hvor VWF-fragmentet indeholder en alaninrest, der er indsat ved substitution i stedet for resterne svarende til resterne 1099 og 1142 af SEQ ID NO: 21.

10. Kimært protein ifølge et hvilket som helst af kravene 1-9, hvor den første Fc-region og den anden Fc-region er identiske.

11. Kimært protein ifølge krav 10, hvor den første Fc-region og den anden Fc-region er afledt fra human IgG1.

12. Kimært protein ifølge krav 8, hvor VWF-fragmentet yderligere omfatter D1-domænet, D2-domænet eller D1- og D2-domænerne af VWF.

13. Kimært protein ifølge krav 12, hvor:
(i) det første polypeptid omfatter en aminosyresekvens, der er mindst 95 % identisk med aminosyresekvensen ifølge SEQ ID NO: 70; og
(ii) det andet polypeptid omfatter en aminosyresekvens, der er mindst 95 % identisk med aminosyresekvensen ifølge SEQ ID NO: 197.

14. Kimært protein ifølge krav 13, hvor:
(i) det første polypeptid omfatter en aminosyresekvens, der er

mindst 99 % identisk med aminosyresekvensen ifølge SEQ ID NO: 70; og
(ii) det andet polypeptid omfatter en aminosyresekvens, der er mindst 99 % identisk med aminosyresekvensen ifølge SEQ ID NO: 197.

15. Kimært protein ifølge et hvilket som helst af kravene 1-14, hvor det kimære protein kan opnås ved hjælp af en fremgangsmåde til fremstilling af det kimære protein, som omfatter
(a) transficering af en værtscelle med et polynukleotid eller et sæt af polynukleotider, der koder for det kimære protein, og b) dyrkning af værtscellen i et dyrkningsmedium under betingelser, der er egnet til ekspression af det kimære protein, hvor det kimære protein udtrykkes,
hvor polynukleotidet eller sættet af polynukleotider omfatter nukleinsyresekvensen ifølge SEQ ID NO: 172, der koder for det første polypeptid.

16. Kimært protein ifølge et hvilket som helst af kravene 1-15, hvor det første polypeptid omfatter aminosyresekvensen ifølge SEQ ID NO: 173.

17. Kimært protein ifølge et hvilket som helst af kravene 1-11, hvor VWF-fragmentet består af D'-domænet og D3-domænet.

18. Polynukleotid eller sæt af polynukleotider, der koder for det kimære protein ifølge et hvilket som helst af kravene 1 til 14 og 17.

19. Vektor eller sæt af vektorer, der omfatter polynukleotidet eller sættet af polynukleotider ifølge krav 18 og en eller flere promotorer, der er operabelt koblet til polynukleotidet eller sættet af polynukleotider.

20. Værtscelle, som omfatter polynukleotidet eller sættet af polynukleotider ifølge krav 18 eller vektoren eller sættet af vektorer ifølge krav 19.

21. Stabil cellelinje, som omfatter værtscellen ifølge krav 20.

22. Farmaceutisk sammensætning, som omfatter det kimære protein ifølge et hvilket som helst af kravene 1 til 11 og 15 til 17 og et farmaceutisk acceptabelt bæremateriale.

23. Kimært protein ifølge et hvilket som helst af kravene 1 til 11 og 15 til 17 til anvendelse til behandling af en blødersygdom eller blødningstilstand hos et individ, der har brug for det.

24. Farmaceutisk sammensætning ifølge krav 22 til anvendelse til behandling af en blødersygdom eller blødningstilstand hos et individ, der har brug for det.

25. Kimært protein ifølge et hvilket som helst af kravene 1 til 11 og 15 til 17 til anvendelse til behandling af hæmofili A hos et individ, der har brug for det.

26. Farmaceutisk sammensætning ifølge krav 22 til anvendelse til behandling af hæmofili A hos et individ, der har brug for det.

DRAWINGS

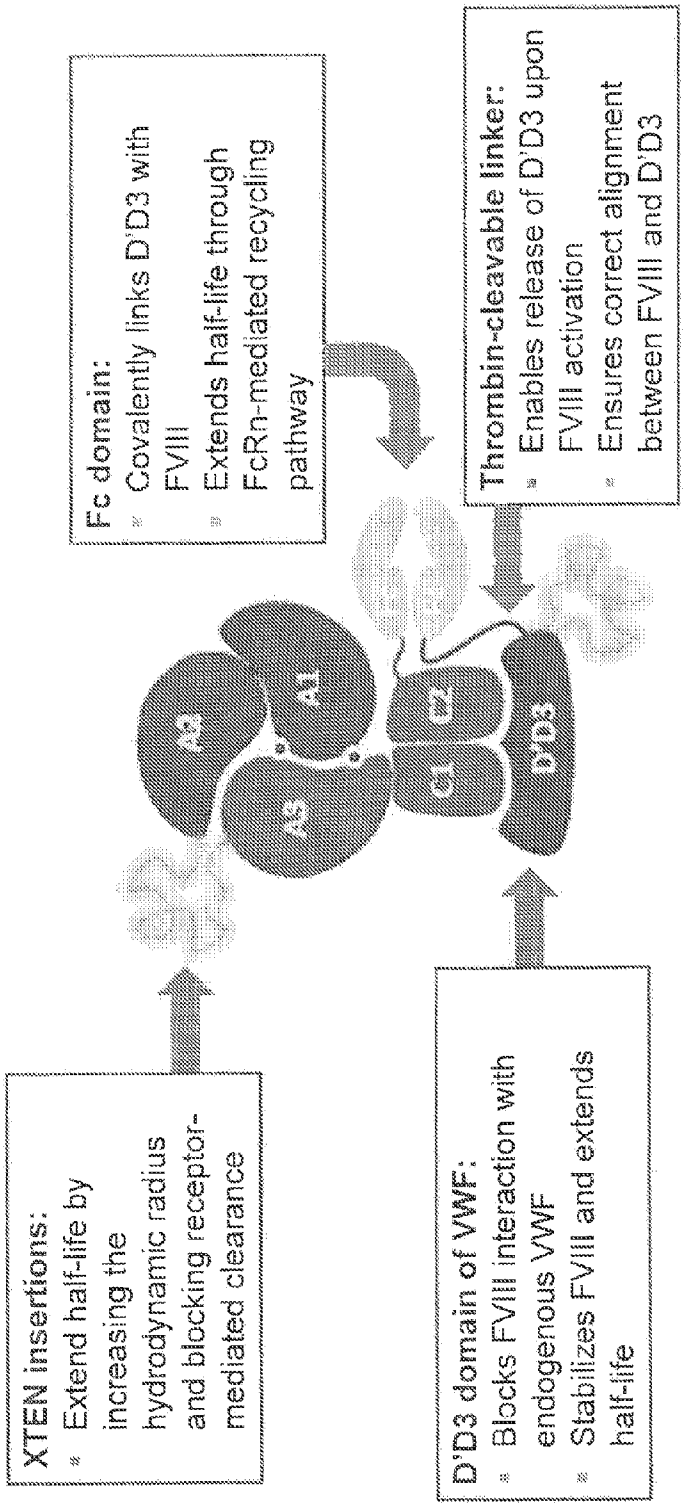


FIG. 1

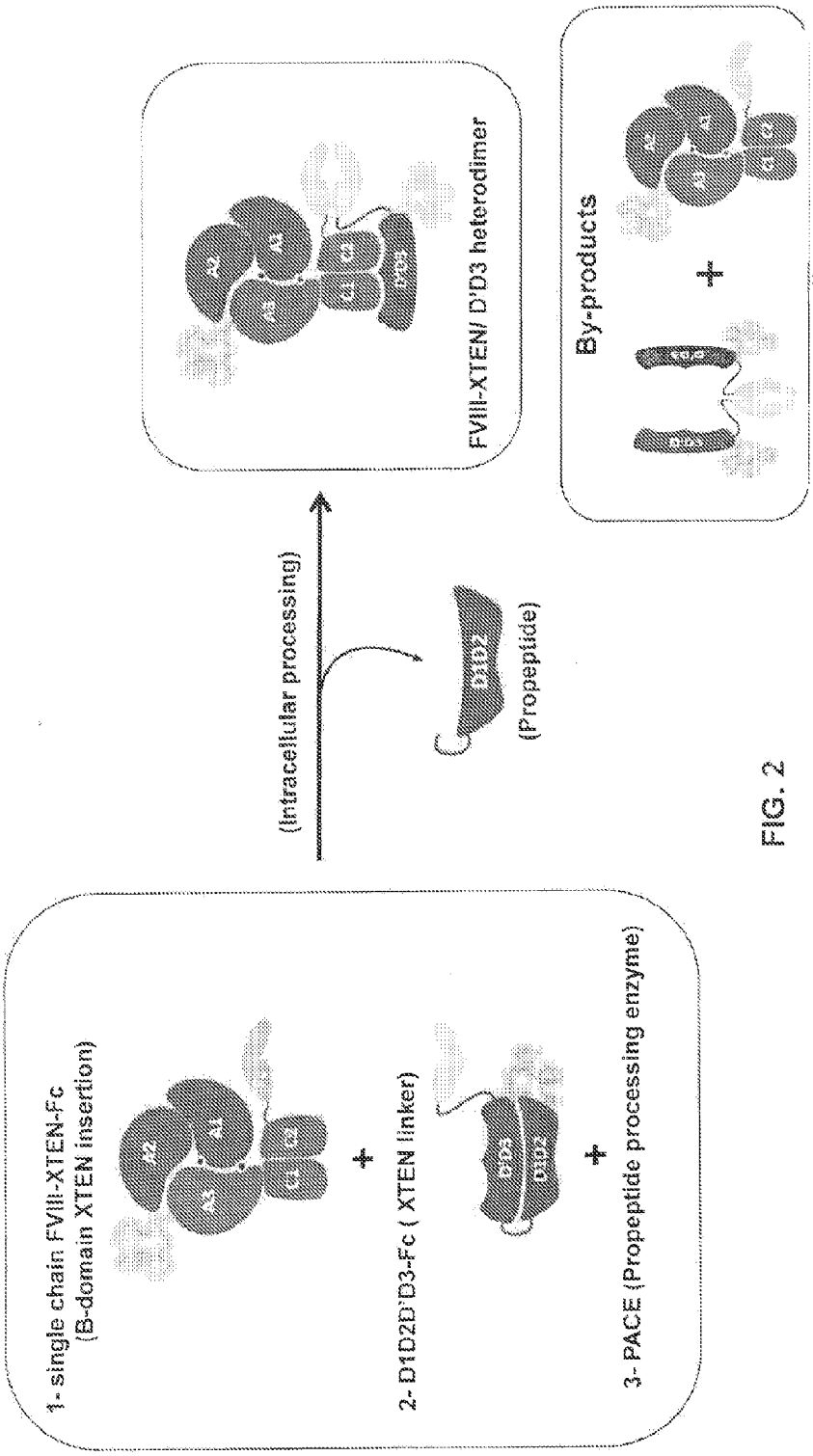

1- single chain FVIII-XTEN-Fc
(B-domain XTEN insertion)
A1
A2
A3
C1
C2
+
2- D1D2D'D3-Fc (XTEN linker)
D'D3
D1D2
+
3- PACE (Propeptide processing enzyme)
(Intracellular processing)
D1D2
(Propeptide)
FVIII-XTEN/ D'D3 heterodimer
By-products
+

FIG. 2

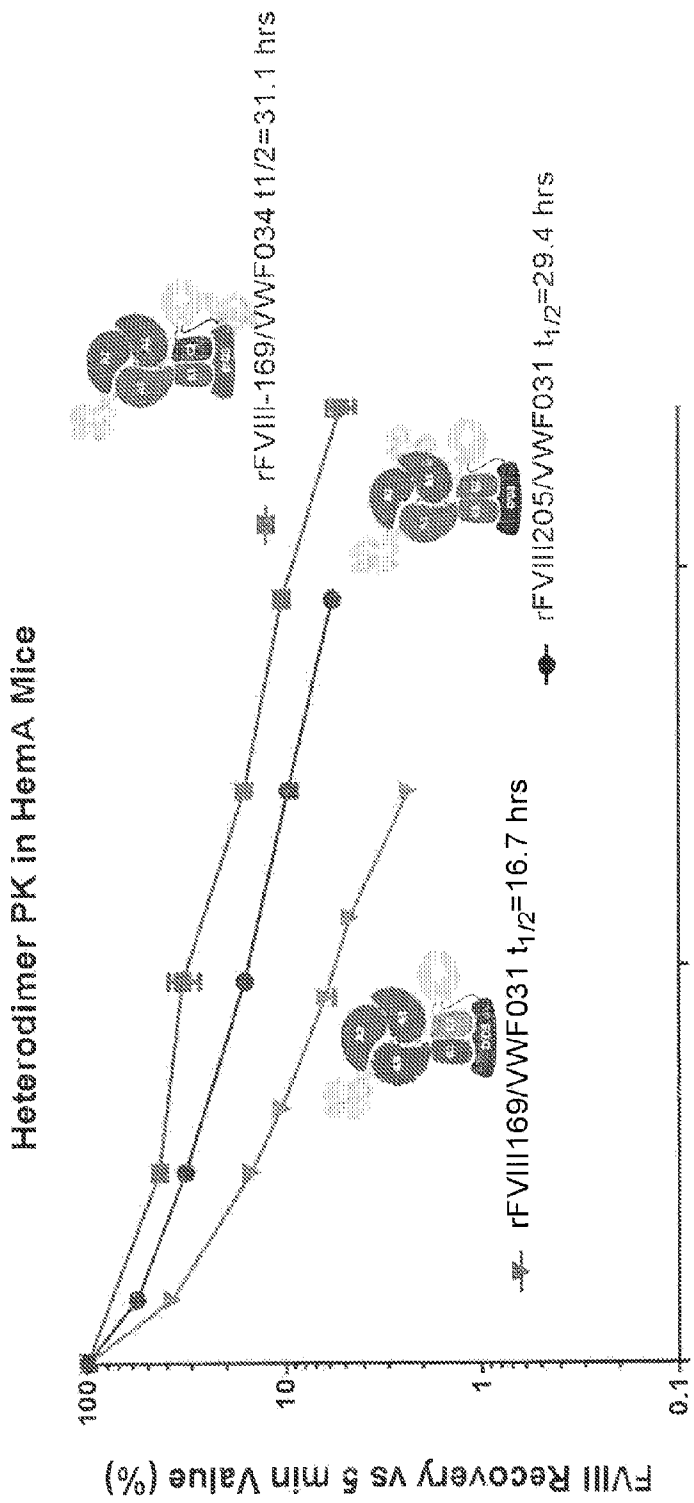

Heterodimer PK in HemA Mice
rFVIII-169/VWF034 t1/2=31.1 hrs
rFVIII205/VWF031 t1/2=29.4 hrs
rFVIII169/VWF031 t1/2=16.7 hrs
FVIII Recovery vs 5 min Value (%)
100
10
1
0.1
0
50
100
Time (Hours)

FIG. 3

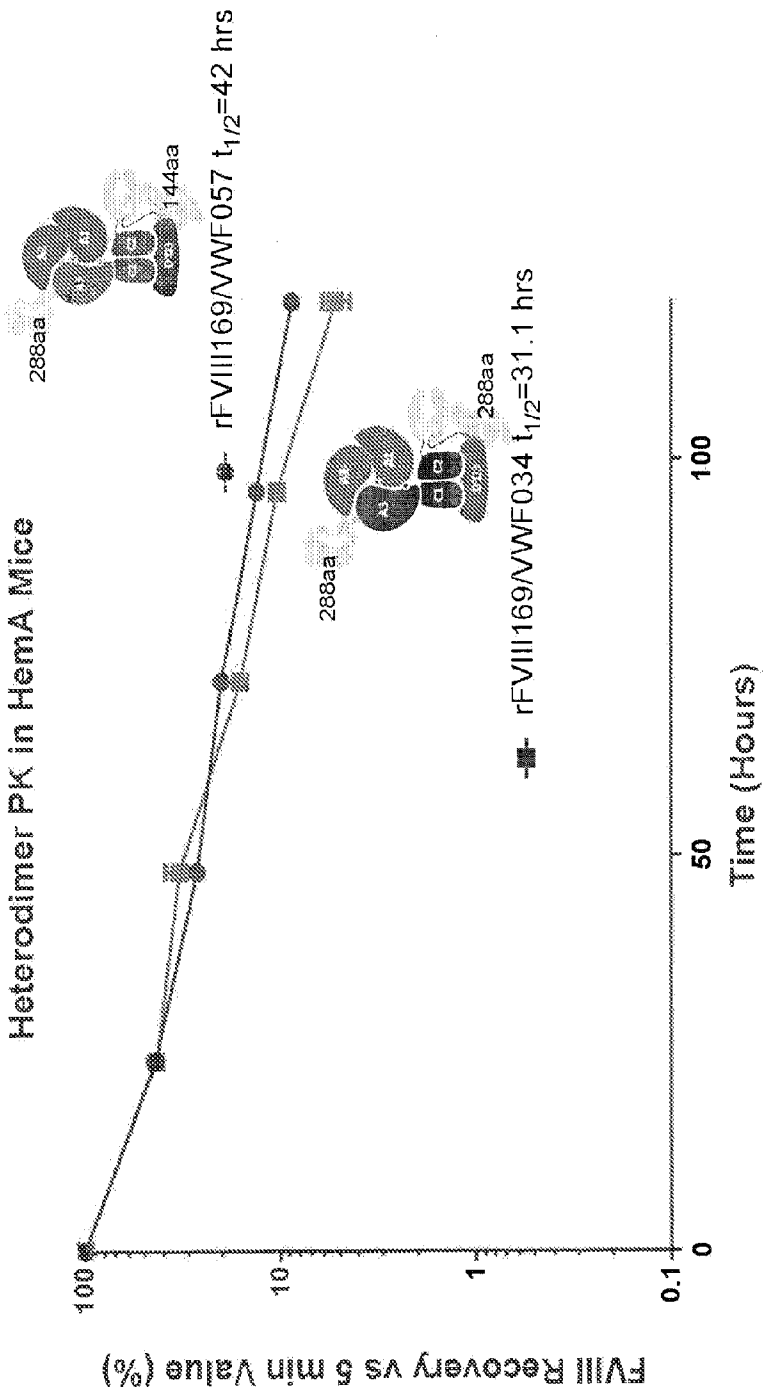

Heterodimer PK in HemA Mice
rFVIII169/VWF057 t1/2=42 hrs
288aa
144aa
rFVIII169/VWF034 t1/2=31.1 hrs
288aa
288aa
FVIII Recovery vs 5 min Value (%)
100
10
1
0.1
0
50
100
Time (Hours)

FIG. 4

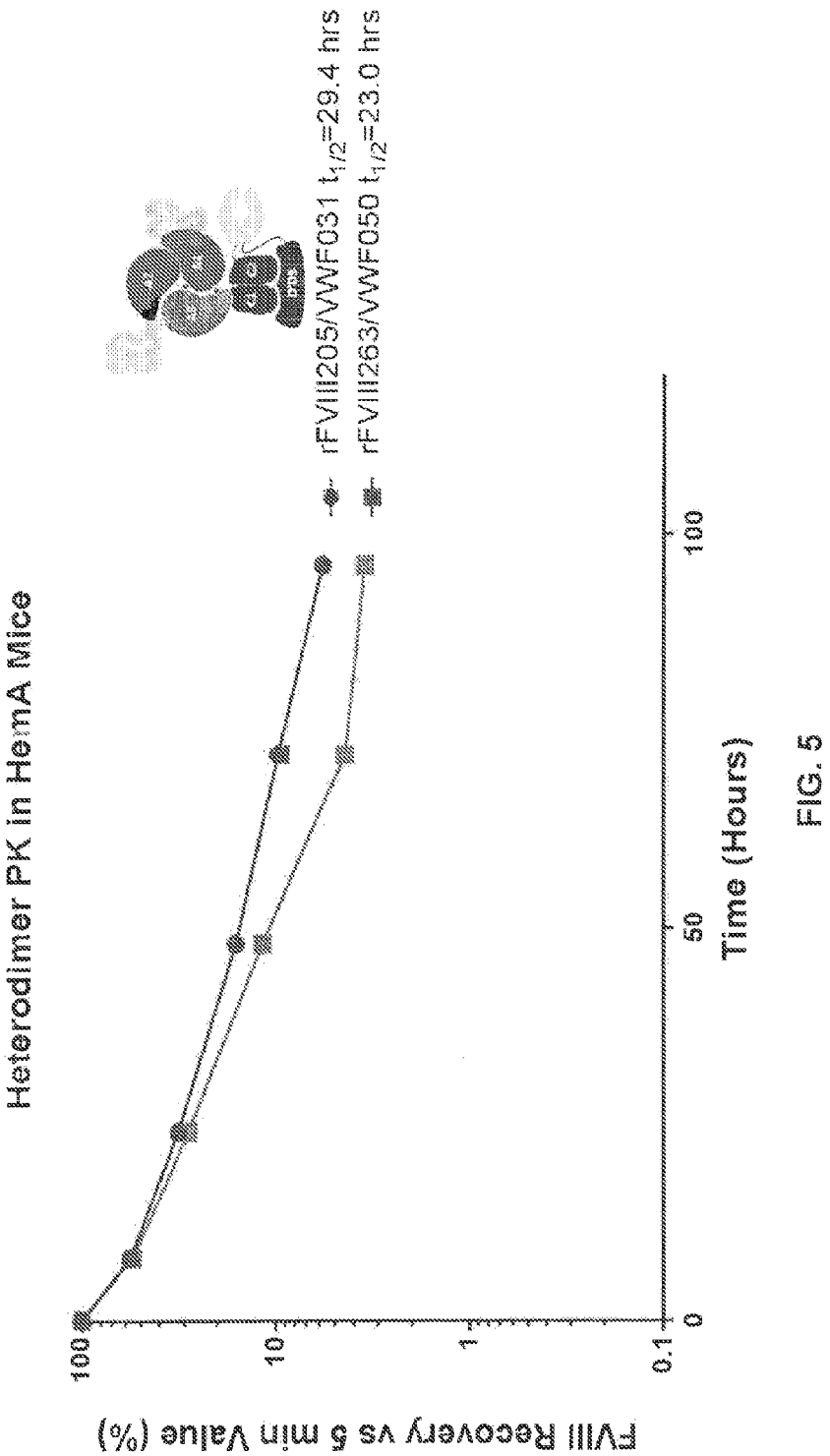

Heterodimer PK in HemA Mice
rFVIII205/VWF031 t1/2=29.4 hrs
rFVIII263/VWF050 t1/2=23.0 hrs
FVIII Recovery vs 5 min Value (%)
100
10
1
0.1
0
50
100
Time (Hours)

FIG. 5

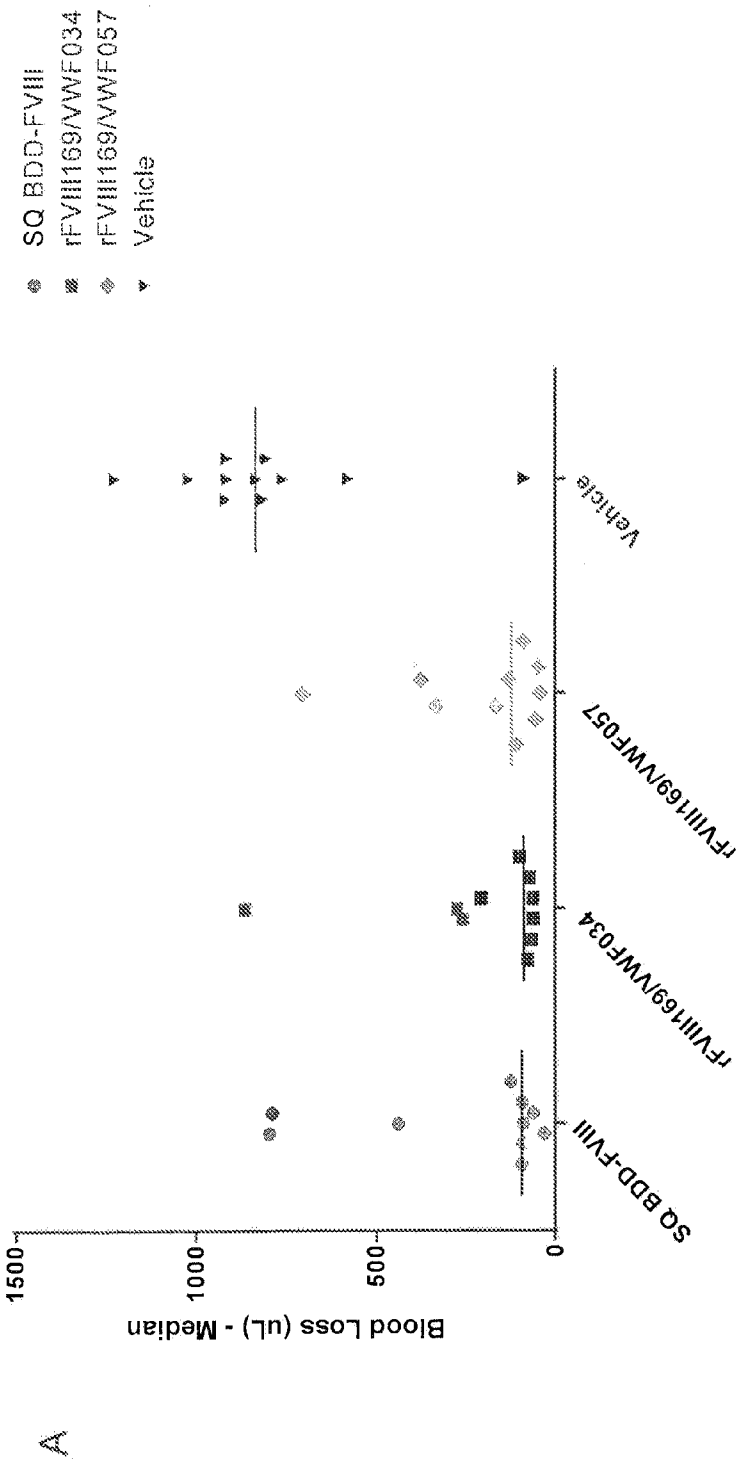

A
Blood Loss (uL) - Median
1500
1000
500
0
SQ BDD-FVIII
rFVIII169/VWF034
rFVIII169/VWF057
Vehicle
SQ BDD-FVIII
rFVIII169/VWF034
rFVIII169/VWF057
Vehicle

FIG. 6

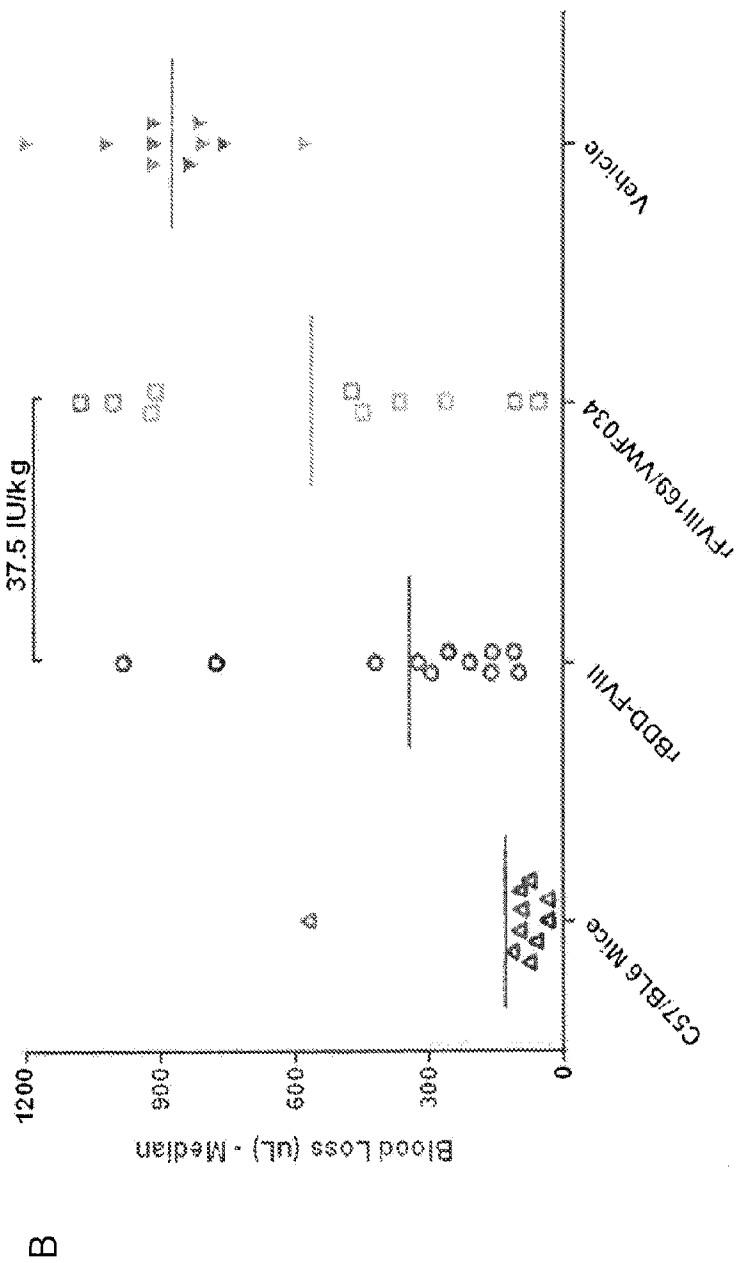

B
Blood Loss (uL) - Median
1200
900
600
300
0
C57/BL6 Mice
rBDD-FVIII
rFVIII-69/VWF034
Vehicle
37.5 IU/kg

FIG. 6

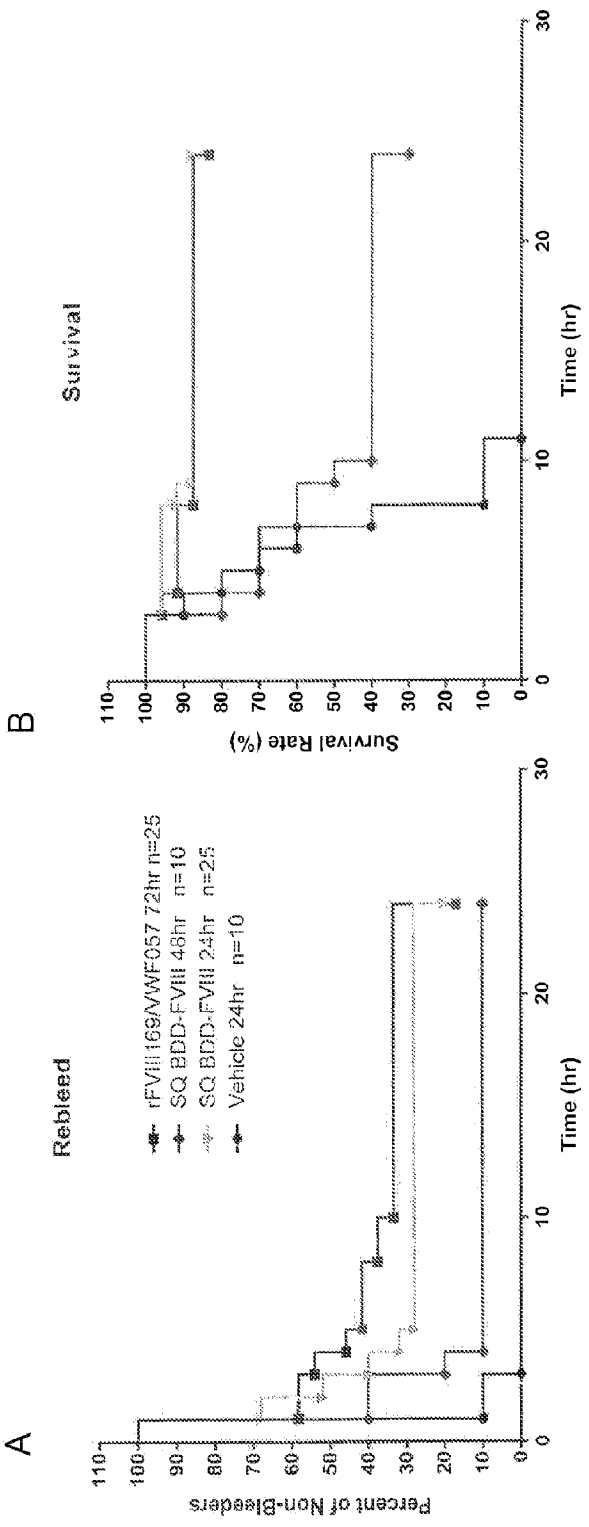

A
Rebleed
Percent of Non-Bleeders
Time (hr)
rFVIII169/VWF057 72hr n=25
SQ BDD-FVIII 48hr n=10
SQ BDD-FVIII 24hr n=25
Vehicle 24hr n=10
B
Survival
Survival Rate (%)
Time (hr)

FIG. 7

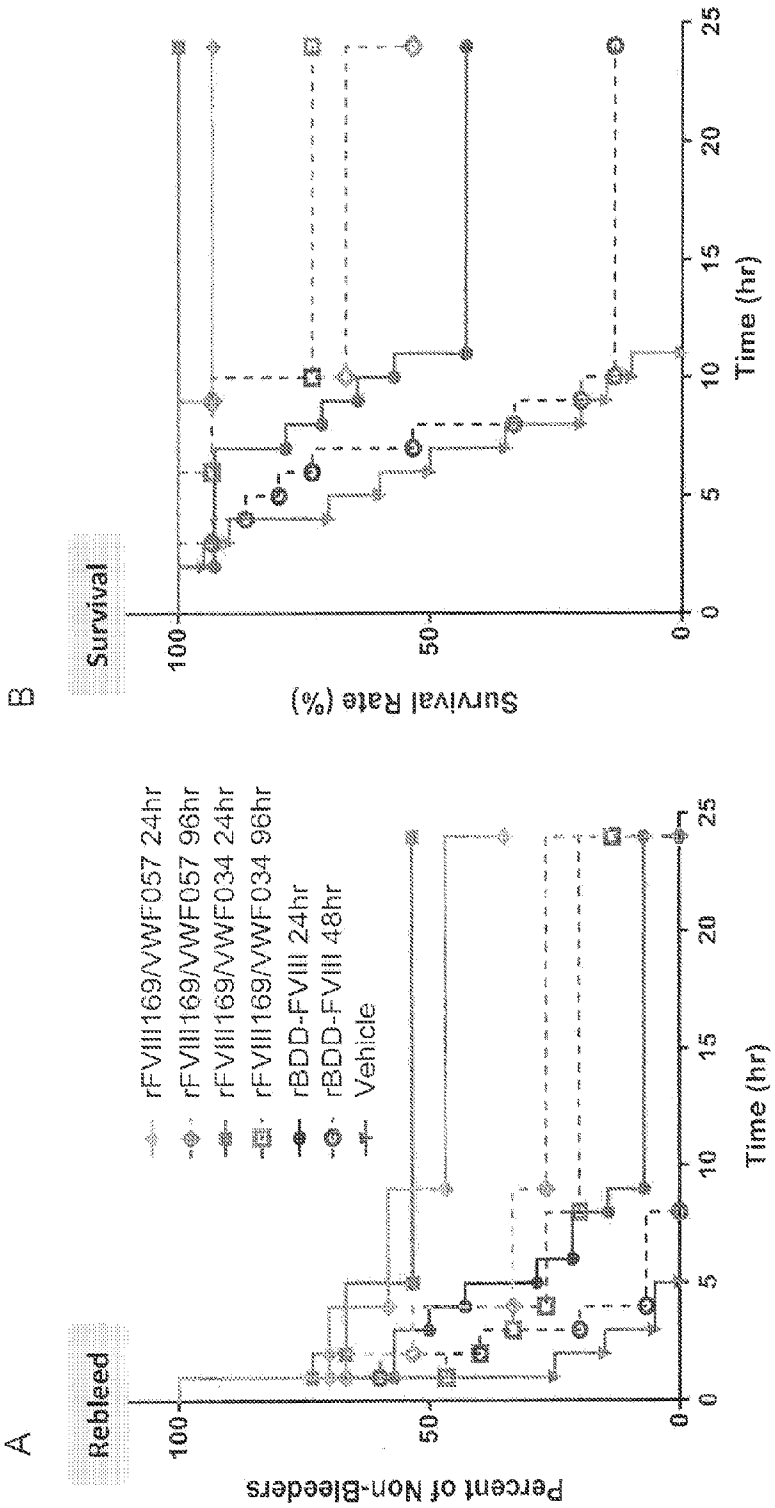

A
Rebleed
Percent of Non-Bleeders
100
50
0
0
5
10
15
20
25
Time (hr)
rFVIII169/VWF057 24hr
rFVIII169/VWF057 96hr
rFVIII169/VWF034 24hr
rFVIII169/VWF034 96hr
rBDD-FVIII 24hr
rBDD-FVIII 48hr
Vehicle
B
Survival
Survival Rate (%)
100
50
0
0
5
10
15
20
25
Time (hr)

FIG. 8

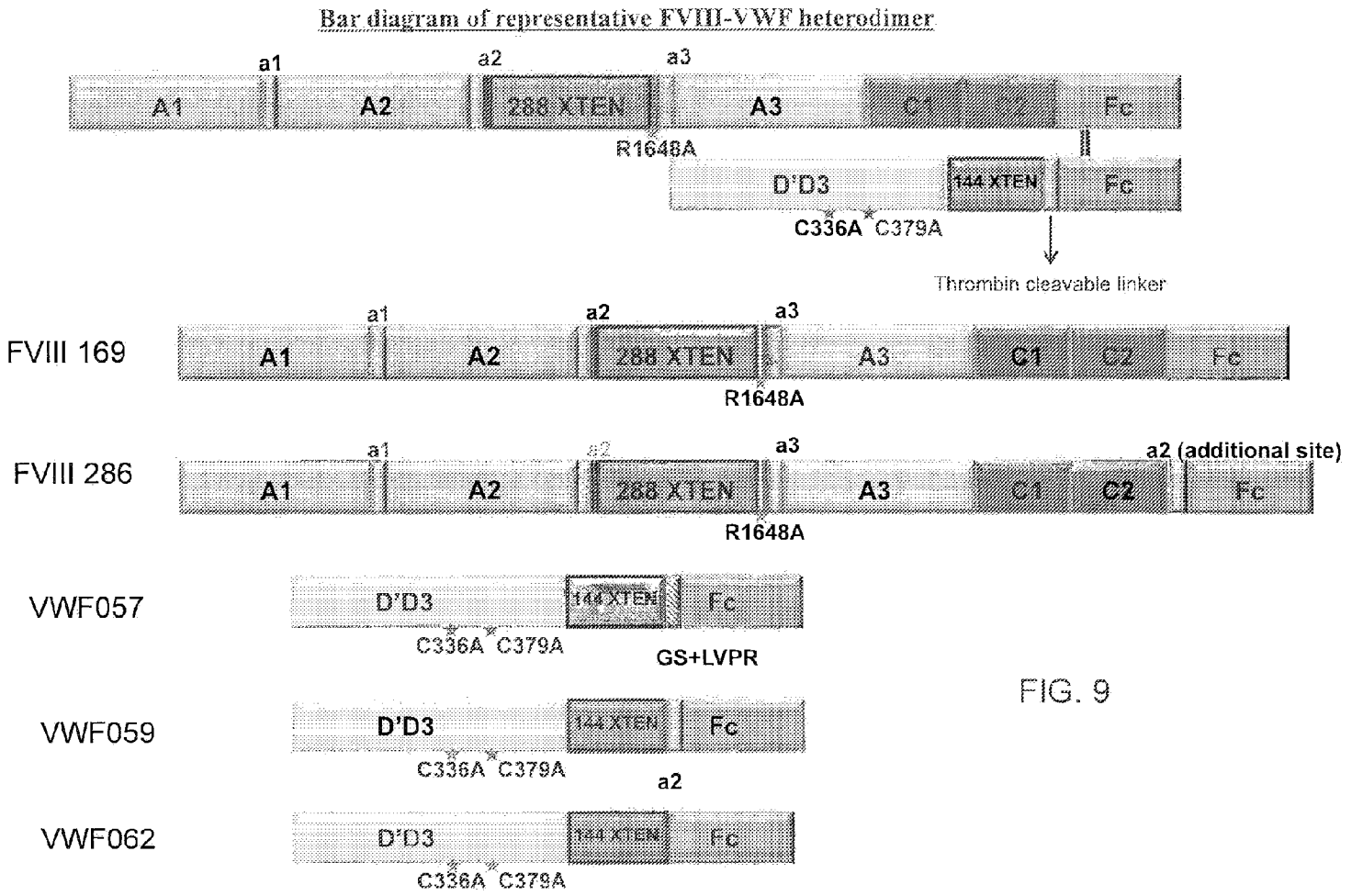
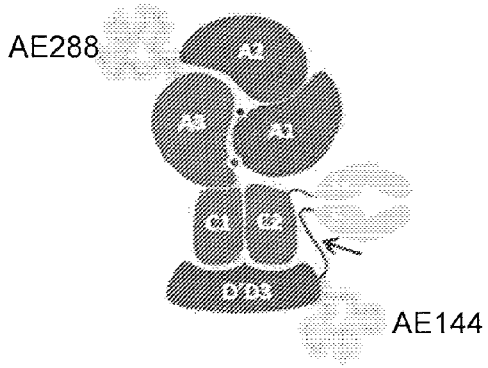


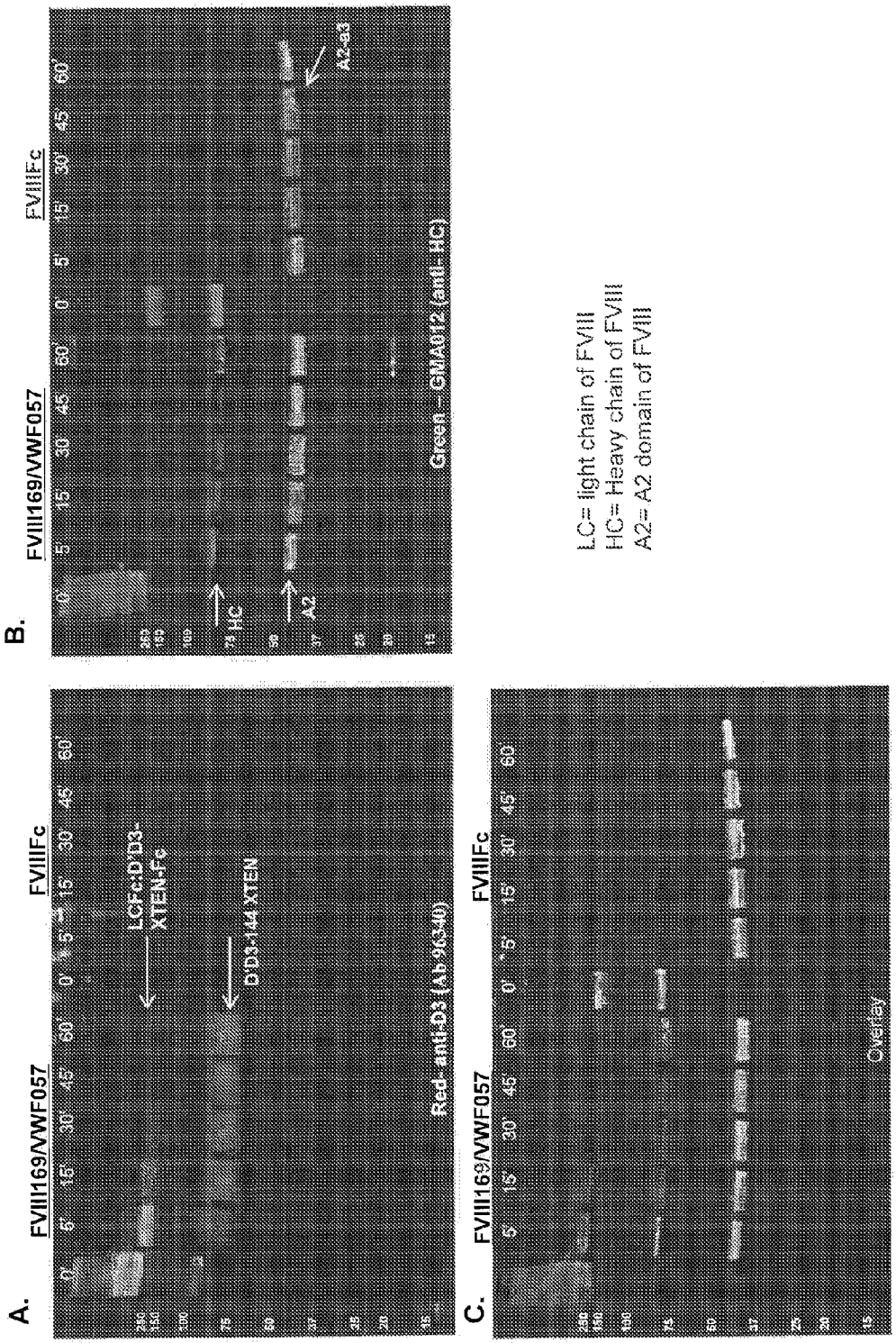
FIG. 9



FVIII169/VWF057 - LVPR site in linker
FVIII169/VWF059 - a2 site in linker
FVIII169/VWF059a - truncated a2 site in linker
FVIII169/VWF073 - a2 fragment in linker

~312 kDa complex

FIG. 10



LC= light chain of FVIII
HC= Heavy chain of FVIII
A2= A2 domain of FVIII

FIG. 11

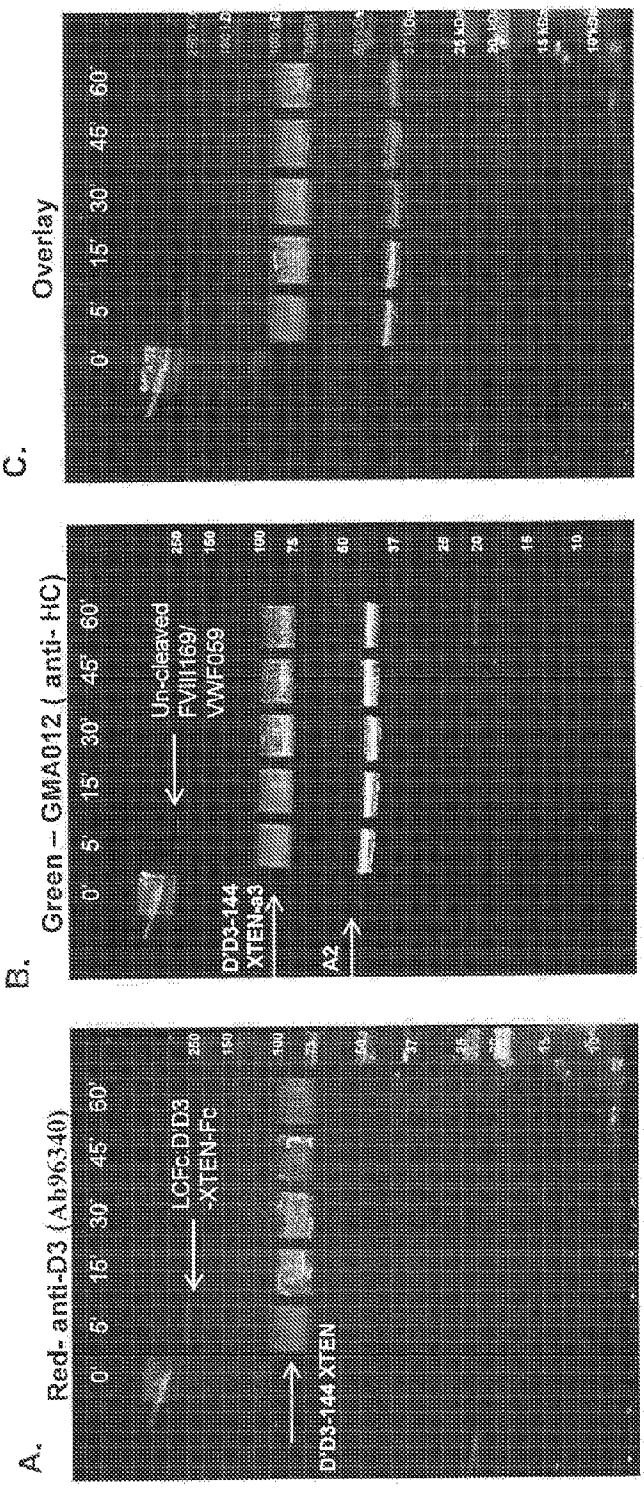

A.
Red- anti-D3 (Ab96340)
0'
5'
15'
30'
45'
60'
LCFc:D'D3 XTEN-Fc
D'D3-144 XTEN
B.
Green – GMA012 (anti- HC)
Un-cleaved FVIII:169/ VWF:069
D'D3-144 XTEN-D3
A2
C.
Overlay

FIG. 12

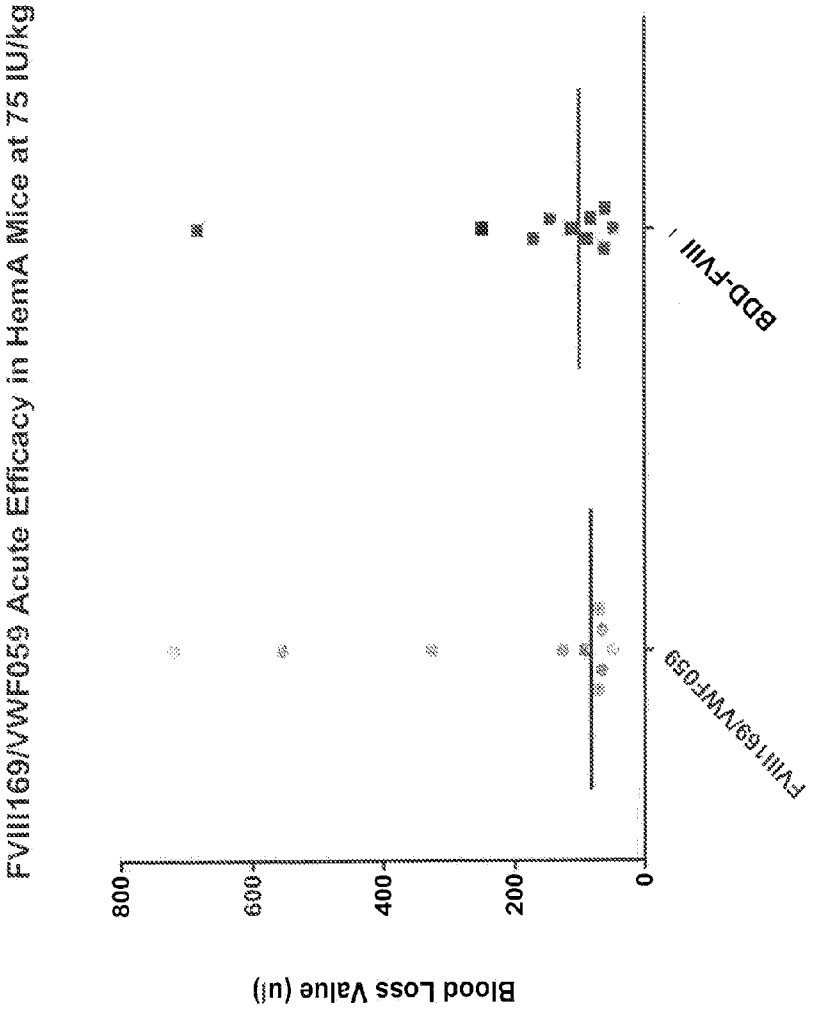

FVIII169/VWF059 Acute Efficacy in HemA Mice at 75 IU/kg
Blood Loss Value (ul)
800
600
400
200
0
FVIII169/VWF059
BDD-FVIII

FIG. 13

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.